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DOCTORAL THESIS

The assessment of the usefulness of the biocybernetic model of a bee colony to predict the effects of giving the queen bee stress associated with space flight

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Podziękowania

Moje najszczersze podziękowania należą się wszystkim, bez których ta praca nigdy by nie powstała:

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Dziękuję.

The Earth is the cradle of humanity, but mankind cannot stay in the cradle forever.
Konstantin Tsiolkovsky

Dagmara Stasiowska

…………………

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Abstract

The dissertation presents the first model of a bee colony, enabling to predict the development of the bee colony with a queen bee subjected to the acceleration pattern of a launching rocket. In the initial chapters, project motivation is discussed, and hypotheses of the model's usefulness are formulated. Additionally, the literature review of available honey bee colony models and past research on space conditions' impact on *Apis mellifera* is presented, showing very limited number of publications available for the topic and presenting various approaches towards model development.

The conducted biological experiment is presented, and acquired results are discussed, presenting the observed impact of rocket launch-related hypergravity on *A. mellifera* queen egg-laying pattern and colony development dynamics. Analysis shows the possible two-fold reaction of the colony. The first scenario assumes the queen maximizes the number of eggs laid with a noticeable increase in the number of drones in the colony. The other scenario contrasts the previous one, limiting the queen in the number of laid eggs, with no significant change in the number of drones in the colony. No other effects were observed, such as the colonies' overwintering readiness.

Assumptions for the model to be chosen are presented, such as the ability to simulate colony development, required simulation time and resolution or the possibility of using real-life observational and weather data as input for the model. Additionally, queen traits changes possibility is presented as a requirement due to the queen's critical impact on the colony development pattern. The BEEHAVE model is presented as the chosen option for further development in the given context due to its wide range of considered factors and fulfilling the criteria mentioned above. New modules are added to its basic version and the new BEEHAVE_spacetravel model is created, enabling simulation of the development of colonies for two space response scenarios observed during the presented biological experiment, named high and low space response scenarios, respectively.

Analysis of the BEEHAVE_spacetravel predictions covers various combinations of the starting conditions and input data, ensuring the model to be thoroughly tested against the gathered real-life data. Performed sensitivity analysis highlights the areas of the greatest impact on the model's prediction correctness, providing additional insight into future research directions.

The obtained results are discussed, leading to general conclusion of the model's usefulness in the given context, with the temporary limitation of its results to specific areas of colony development. However, this state of the matter and other minor limitations of the model may change in the future once more biological research results on the impact of hypergravity on bee colony development are available.

Streszczenie

W rozprawie przedstawiono pierwszy model rodziny pszczelej umożliwiający przewidywanie rozwoju rodziny pszczelej w której matka została poddana przeciążeniom związanym ze startem rakiety. W początkowych rozdziałach omówiona zostaje motywacja projektu oraz formułowane są hipotezy dotyczące przydatności modelu. Dokonany przegląd literatury na temat dostępnych modeli rodziny pszczelej i dotychczasowych badań nad wpływem warunków kosmicznych na pszczoły miodne (*Apis mellifera*), pokazuje bardzo ograniczoną liczbę dostępnych publikacji na ten temat i przedstawia różnorodność podejść w zakresie rozwoju modeli.

Przeprowadzony eksperyment biologiczny wraz z uzyskanymi wynikami zostają omówione, prezentując zaobserwowany wpływ przeciążeń na liczbę i dynamikę składania jaj przez matkę pszczelą oraz ogólną dynamikę rozwoju rodziny pszczelej. Analiza pokazuje dwojaką reakcję kolonii: pierwszy scenariusz zakłada, że matka pszczela maksymalizuje liczbę składanych jaj przy dodatkowym, zauważalnym wzroście liczby trutni w rodzinie. Drugi scenariusz, będący w opozycji do poprzedniego, wykazuje ograniczenie matki w liczbie składanych jaj, bez znaczącej zmiany liczby trutni w kolonii. Nie zaobserwowano żadnych innych efektów, takich jak zmiana w przygotowaniu rodzin do zimowli.

Przedstawione zostają założenia modelu, takie jak zdolność do symulacji rozwoju rodziny pszczelej, wymagany czas i krok symulacji czy też możliwość wykorzystania rzeczywistych danych obserwacyjnych i pogodowych jako plików wejściowych do modelu. Dodatkowo, z uwagi na istotny wpływ matki pszczelej na dynamikę rozwoju rodziny, możliwość zmiany liczby składanych jaj przez matkę pszczelą zostaje przedstawiona jako dodatkowy wymóg. Model BEEHAVE zostaje wybrany do dalszego rozwoju w zadanym kontekście, ze względu na szeroki zakres uwzględnianych czynników oraz spełnianie omówionych kryteriów. Do podstawowej wersji modelu zostają dodane moduły umożliwiające symulację rozwoju rodziny pszczelej dla dwóch zaobserwowanych reakcji matki pszczelej na poddanie jej przeciążeniom. Moduły zostają nazwane odpowiednio scenariuszem wysokiej i niskiej odpowiedzi na warunki kosmiczne, a nowy, rozszerzony model otrzymuje nazwę BEEHAVE_spacetravel.

Analiza prognoz modelu BEEHAVE_spacetravel obejmuje różne kombinacje warunków początkowych i danych wejściowych, zapewniając rzetelne przetestowanie modelu pod kątem zebranych danych rzeczywistych. Przeprowadzona analiza wrażliwości modelu podkreśla obszary o największym wpływie na poprawność prognoz modelu, stanowiąc jednocześnie sugestię dla przyszłych kierunków badań.

Uzyskane wyniki zostają omówione, udowadniając przydatność modelu w zadanym kontekście, z tymczasowym ograniczeniem dokładności jego wyników do określonych obszarów rozwoju rodziny pszczelej. Ograniczenie to jednak, jak również inne pomniejsze aspekty poprawności prognoz modelu, najprawdopodobniej ulegną dezaktualizacji w przyszłości, kiedy zostanie przeprowadzona większa liczba badań nad wpływem przeciążeń na rozwój rodziny pszczelej.

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1. Introduction

The 20th of July 2024 marks the 65th anniversary of the first human being to set foot on another celestial body in the history of humankind. After this historical event, many things in the human world have changed, except for the human urge to explore. The exploration is fueled by bold visions of settling on the Moon and Mars. To achieve this dream, various space agencies, companies, and organizations join forces and pursue research in all the areas that will be critical for future extraterrestrial settlers – from rocket engine development through radiation and altered gravity impact on human body studies to complex life support system designing.

One of the components of the life support system is food production, providing astronauts with fresh food full of necessary nutrients. Freshly grown fruits and vegetables may appear to not only be a natural source of vitamins and minerals but also, what might be of even greater importance, may serve as a link to nature and Earth. Many astronauts onboard the ISS declare that plant-growing experiments are their favorite, lowering work-related stress and giving them a sense of meaning [1], [2], [3], [4], [5], [6]. Such an effect should not be neglected, as one of the hardest parts of long duration space travels will most likely be a feeling of closeness caused by living in a small, restricted space and loneliness.

Due to the limited resources of any kind during a space travel, currently developed food production systems for space applications, in most cases, consider usage of hydroponic or aeroponic cultivation method, due to their low water demand. Many other aspects of plant development are being researched, such as the impact of lower gravity [7], [8], [9], [10], artificial lighting [8], [11], soil/nutrient composition [12], [13], [14], [15], [16] on plants growth potential. One of the least investigated topics is the matter of pollination. While on Earth, this particular topic usually does not have to be considered on the first place, in the case of a fully enclosed "artificial" system it starts to be critical to take it into consideration.

There are several major methods of plant pollination: wind pollination, cross-pollination, self-pollination, animal pollination, and combinations of those. Animal pollination, while not crucial for many species, was proven to enhance fruits traits, such as quality and storability [17], [18]. That might appear crucial in the case of extraterrestrial crops, where the maximization of yield will be of great importance. Additionally, the Food and Agriculture Organization of the United Nations claims that close to 75% of the world's crops, producing fruits and seeds for human consumption, depend, at least to some extent, on pollinators [19]. Traditional meat production is very resource-demanding, for which reason most probably it will not be performed on the surface of the Moon or Mars. In such case, many important micro- and macro-elements, such as zinc, magnesium, or potassium, should be provided in a different manner. A plant-based source of them is, for example, sunflower [20], [21] or pumpkin [22] seeds – those, however, strongly depend on proper, insect-induced, pollination. For the following reasons, the subject of animal-induced pollination should be investigated more carefully.

Western honey bees (*Apis mellifera*) are associated mainly with honey production. However, due to the decline in the number of wild pollinators, increasingly more regions rely on their pollination services [23], [24], [25]. The clearest examples are the great almond plantations in California, requiring approximately 60% of commercially managed honey bees in the U.S. to be transported to the area during flowering season [26]. Although the species is not the only one used in crop pollination [27], [28], their long history with humankind and possible positive impact of beekeeping on mental health and coping with isolation [29], [30] make them the first choice for extraterrestrial pollination-based food production.

From the beginning of modern beekeeping, honey bee biology and behavior were studied and described extensively. Phenotypical and behavioral plasticity of honey bees led to the use of the species as a model organism in the broad area of science [31]. The same plasticity makes *A. mellifera* an ideal candidate for pollinating extraterrestrial crops.

Any living object to be transported to another celestial body needs to survive space travel, particularly rocket transportation. The flight is known to consist of two violent stages, generating significant increases in gravity value - launch and re-entry. For this reason, the verification of the ability to survive a rocket flight-related hypergravity in a condition enabling further development and reproduction should be the first stage of the assessment of the suitability of a species for extraterrestrial crop pollination.

However, establishing the impact that space travel could have on honey bee colony development requires perennial observations, generating a sufficient amount of data to conclude on the topic. From a close perspective of extraterrestrial settlement, there is a need to verify it in a shorter time than normally required. An answer to the limited data and time to conclude space travel's impact on honey bee colony development might lie in computer modeling [32], [33]. Using such, the long-term effects could be assessed by simulating the required period of life of a bee colony with a queen given previously to the stress related to the rocket launch and later by comparing obtained results with the healthy, Earth-based colony. As previously shown by some of the existing models, the egg-laying rate of the queen has the biggest impact on the colony development [34]. The second most important factor indicated by the mentioned model was the queens' availability to spermatozoa. In the case of the queen subjected to high hypergravity values, this aspect could be impaired, e.g., by damage to the spermatheca duct or another part of the reproductive system. As suggested by Pettis et al. [35], low sperm viability is linked to colony performance.

A similar modeling approach is used during studies on the impact of various environmental stressors on bee colony development [36], [37], [38], [39], [40], [41]. Additionally, modeling enables the consideration of further factors important for colony survival and correct development, such as the availability of food sources and their nutritional value, weather, or daylight presence.

Nevertheless, the use of a computer model will be strongly limited by its existence and usefulness in the context of research. Therefore, there is a need to develop a model enabling the simulation of the honey bee colony in which the queen experienced rocket launch-related hypergravity. Moreover, such a model shall be suitable for further development in extraterrestrial bee usage, e.g., by expanding it with new parameters describing the impact of newly researched factors. The proposed approach would accelerate the process of establishing a fully functional, low-maintenance extraterrestrial greenhouse where crops could be naturally pollinated and would not require the assistance of energy-demanding equipment. Such an improvement could be further used not only beyond Earth but also implemented in Earth-based solutions, enhancing crop yield in greenhouses and supporting local food production in isolated regions.

2. Thesis – the goal

The goal of the thesis is to verify if, and if so, to what extent, the existing computer model of the bee colony BEEHAVE can be adapted to be used for the prediction of the development of the western honey bee (*A. mellifera*) colony in which the queen was given to the acceleration of a launching rocket. Hence, in the presented thesis, the following hypotheses were tested:

H1. With appropriate updates, the BEEHAVE model is capable of simulating the development of a bee colony with a queen subjected to hypergravity.

Equipping the BEEHAVE model with experimental data and updating some of its parts to meet the specific requirements of the research enables simulation results in line with real-life measurements. Results are true for more than one parameter simulated, such as number of eggs laid throughout the season, amount of food stores, and colony or queen death.

A simulation can be a tool for the long-term assessment of the honey bee colony development dynamics, enabling the prediction of possible deviations in relation to the healthy colony. In the future, when such a model is developed and proven to be reliable, simulation of the development of the altered colony (with queen bee influenced by the hypergravity appearance) will enable to e.g., predict its failure and implement actions preventing it. Until then, due to the limited data on the hypergravity impact on honey bee colony development, the model could be used to predict possible changes in the development process and to highlight the aspects of such biological research that should be particularly considered.

An additional hypothesis was also formed in the case of the BEEHAVE model being unable to fully predict hypergravited honey bee colony development:

H2. The BEEHAVE model can be used to simulate specific parts of honey bee colony development, such as honey production or honey bee queen life span prediction, for the colony in which honey bee queen was given the acceleration pattern of launching a rocket.

The hypothesis assumes that specific parts of a bee colony's development dynamics might be correctly simulated despite the failure to simulate all the aspects. Specific features may involve e.g., food stores amount, number of eggs throughout the season, colony death, or queen death.

3. Background

Due to the specifics of the research subject, the literature analysis has been divided into two parts: first, it describes existing knowledge on the matter of space conditions' impact on pollinators, with an emphasis on bees and honey bees in particular. The second part of the literature analysis focuses on available models of bee colonies, analyses them in the context of considered aspects of honey bee colony life and indicates which one of them is the most suitable in the researched context.

Literature search for both topics was conducted in an analogous manner – the Google Scholar, PubMed, and Web of Science databases were searched using several keyword combinations. Additionally, it was completed by a separate search through the reference list of the most relevant publications. For bee colony modeling, all the publications on artificial bee colony algorithm (ABC) were excluded as being mainly used as an optimization algorithm and not a simulation of real-life honey bee colony development *per se* [42].

Keywords used for the bees in space literature search consisted of: bee, honey bee. honeybee, space, microgravity, bumble bee, pollinator, pollination.

Keywords used for the bee colony model literature search: bee, honeybee, honey bee, model, simulation, colony, population.

3.1. Space travel impact on bees

There is a record of several bee species that have been sent into space by NASA. In 1984, a study on survival, behavior, and comb construction in microgravity [43] was conducted. The B.E.M. (Bee Enclosure Module), a dedicated compartment enabling live observation of honey bees on board the space shuttle *Discovery* was a part of the STS-41 mission. A colony composed of around 3,400 specimens (0.45 kg) and one queen took a trip into space, experiencing a maximum acceleration of 3 g during the shuttle launch. During its stay in space, the queen laid approximately 35 eggs. However, this study is burdened with a serious methodological flaw, as there is no data on the number of eggs laid at the same time by the control queen, which was left with its colony of the same size in Johnson Space Centre in an analogous module and centrifuged to simulate launch and ascent conditions [44]. After the return of the shuttle, both samples were transferred to a standard hive, where all the space eggs failed to hatch. What is more, approximately 120 worker bees died in the space sample and 350 – in the ground one [43]. Such a result might suggest a negative impact of hypergravity alone on honey bee workers' survivability and lifespan.

Such a hypothesis is supported by the results of another experiment performed in 2011 by scientists from the Institute of Apicultural Research (IAR), Chinese Academy of Agricultural Sciences (CAAS). The experiment's aim was to use space technology and conditions to create an excellent variety of beneficial traits for bees. The study showed that the honey bee drone's semen viability decreases significantly after a 2 to 3-week-long stay in space onboard the satellite. The longer the time in orbit, the higher the decrease in sperm survival rate. Additionally, the lifespan of the queen bee inseminated with such semen also decreases – for 30 queens inseminated with "space semen", the mortality rates of queens within one week were 50.0% and 78.9%, respectively for two and three-week stay in orbit, while for 20 queens from the control sample, mortality rates were 24.8% and 22.4% [45], [46]. What is more, the ability of queens to lay diploid eggs was strongly affected, with the increased rate of laying unfertilized eggs. While the first offspring generation did not show abnormalities in the morphology, the second and the third showed increased presence of deformities such as the residual wings, back plate, and abdomen deformities, with no such event for the control group specimens.

Another NASA research involving bees, performed in 1982, investigated the flight pattern of honey bee workers, moths, and houseflies in microgravity and checked their ability to adapt to such conditions [47].

Fourteen honey bees were chosen for the experiment in 0 g and twelve workers for the 1 g control. All specimens were approximately six days old and had their stingers "clipped", to ensure the mission's crew safety. Honey bees, along with moths, were unable to control the direction of travel when in flight, which resulted in collisions with other insects. Flies, most probably due to halteres enabling active flight direction control, were able to control their orientation and flight paths better than the rest of the species. All 14 worker bees from the 0 g sample died before returning to Earth. In the 1g sample, five were alive after the experiment ended. Post-flight analysis of bees showed no damage to wings, legs or bodies. Additionally, it proved that specimens were free from any bee disease and that clipping of specimens' stingers had no impact on their lifespan. What most probably had a negative impact on the bees' lifespan was the sugar solution provided during the mission, being insufficient for their needs and able to sustain them for no longer than nine days. The total mission time was eight days.

Another experiment was aimed to be conducted in 2003 and was a part of school engagement in the space shuttle program. Its aim was to study the ability of carpenter bees (*Xylocopa*) ability to construct nests in microgravity conditions. However, the experiment was lost due to the fatal crash of the space shuttle upon re-entry. Nonetheless, the above projects suggest that when fed properly, pollinators could function normally under microgravity conditions during long-term space travel. However, to verify this, more studies are needed.

In 2019, an experiment on near-space conditions' impact on *A. mellifera* queens was conducted [48]. Three sister honey bee queens with attendants were placed onboard the gondola of stratospheric balloon along with sensors measuring data on radiation, UV-C level, temperature, and pressure. Three sister queens were left on Earth as an experiment control. The flight was 134 minutes long, and gondola was successfully recovered ~9 km away from the launch site. All specimens hibernated during the flight due to the significant drop in temperature. Most probably due to the harsh weather conditions on the experiment day, all queens from test and control samples suffered from the significant drop in temperature, which, as a result, caused their deaths within the next two weeks after the experiment ended.

The same year, the *BeeGs* project started with a series of experiments with honey bees on board the sounding rockets [49]. The first experiment was conducted with the *Carbonara* – sounding rocket, reaching a maximum altitude of approximately 1 km and a maximum acceleration of 3 g. Seventeen worker bees were placed in the dedicated testing device onboard the rocket, and 15 workers were placed in the same device as a control sample. All the specimens survived the flight in good condition, initially proving *A. mellifera* ability to survive significant acceleration increases.

The other experiment of the project was conducted in the US on a group of 35 worker honey bees in total [33]. Bees were placed in the experimental device called *BeeO!Logical* for three subsequent days due to the complications in acquiring the launch permission. When the permission was acquired, 11 out of 19 workers of the test sample and 13 out of 16 workers of the control sample were still alive and qualified for the experiment. The *PROtotype* sounding rocket was expected to reach an altitude of 3 km; however, due to one of the subsystem's malfunctions, it experienced substantial flight path change and reached the apogee of only 225 meters with a maximum total acceleration of 6.5 g. the survivability of the workers which qualified for the final flight was 54.5% for the test sample and 100% for the control sample. Such a result might imply the negative impact of the acceleration on honey bee survivability rate.

The data focusing on *A. mellifera* in the context of space travel is scarce, and despite a thorough literature query, no more information was found. None of the research considered directly the queen's fecundity or fertility. This aspect is essential as the use of bees as pollinators on another celestial body can only be considered if the queen is able to reproduce and produce viable offspring and, therefore, sustain the colony.

3.2. Computer modeling of a honey bee colony

3.2.1. Introduction

Modeling is a powerful tool broadly used by scientists of various fields and backgrounds. It enables the synthesis of numerous sources of information [50] and improves an understanding of the functioning rules of a specific system [51]. Another application is the predictive analysis and decision-making support [52].

Generally understood models seem to be particularly useful in biology-related issues – problems' complexity and range of factors to be considered make it often impossible to be solved by other, less advanced techniques [53]. In the medical field, models are helpful for doctors to establish the amount of the medicine necessary to cure a patient [54], [55] or to check what may be the possible side effects of cross-usage of two substances [56], [57].

At the same time, simplification of analyzed processes, required by many modeling tools, might help to improve their understanding. Additionally, it does not require as high computing power as the neural network or other AI-based systems. For that reason, modeling is a popular tool widely used by biological and medical sciences.

One of the highly complex biological systems which have been modelled is a development of a colony of honey bees. Such models have been developed since the 1980s, and now, many different approaches to the topic, as well as various scopes of the researched aspects, can be found. Some of them are used for a better understanding of processes that take place inside the hive [58], [59], while others are studied by public institutions to assess if they could be a tool used in a regulatory and legislation-making context [37].

A model can simulate a couple of years-long development of an entire bee colony and execute such a simulation in a matter of seconds. Thanks to that, various environmental conditions and their impact on colony development and survival chances can be verified. Data describing the egg-laying behavior of the honey bee queen after experiencing the rocket-launch-related acceleration could, therefore, be used to determine the chances of survival of the rocket-transported colony. What is more, the possibility to additionally verify the impact of various external conditions, including parameters such as final food source composition or specific weather conditions, opens more possibilities than the regular experiment.

Existing models of a bee colony can be divided into two main groups: modeling some specific aspect of a bee colony life and considering possibly all the aspects relevant to the colony. The first group usually investigates the modeled topic in more detail, while the other one, when considering most of the aspects, does not look at its very details but enables it to focus on the "bigger picture".

All the available and described honey bee colony models in the literature were analyzed and divided, as mentioned above, into two categories named respectively "partial" and "holistic".

3.2.2. Category I: Holistic models

The first works on creating a modeling tool for honey bee colonies are dated to the late 1970s [60] and early 1980s [40], [61], [62]. At that time, one of their main purposes was to help beekeepers to simulate bee colony development and to predict pollen and honey production. The model was created at AGH University of Krakow in Poland by Migacz and Tadeusiewicz [60] and considered ten major aspects of honey bee colony life: reproduction, food collection, economy and consumption, honeycombs building, searching, in-hive information exchange, environmental conditions, weather and beekeeping activities. It used FORTRAN to describe algorithms, and the output of the model was presented as 2-d and 3-d plots of the parameters. Its possibilities were discussed during beekeeping conference in Puławy in 1988 and full source code using BASIC language was published [63]. This model is also described as the “(bio-)cybernetic”. Due to their age, they did not consider the presence of *Varroa destructor* in the hive and its impact on the survivability of a colony.

In the late 1980s, the BEEPOP model was also created [34]. It simulated bee colony population dynamics and could be used for environmental risk assessment. Due to the models’ creation time, similar to the Polish model, the group in the initial phase used FORTRAN software for operation; later, a version dedicated to IBM personal computers was created [64], [65]. In 1990, it was equipped with an additional component, enabling it to determine the colony’s genetic composition [66]. The basic BEEPOP version consisted of two major sections: the first one focused on the queen’s fecundity in time, and the other analyzed eggs’ development and foraging activity with consideration of the weather data.

Based on that solution, in the 2020s, the US Environmental Protection Agency (USEPA) and the US Department of Agriculture (USDA) developed the BeePop+ [67], a simulation model assessing potential honey bee colony-level effects of pesticides. The model can be used for various US locations, and its results are consistent with the observational data. However, the need for additional evaluation is highlighted by the authors.

Another model that enabled the consideration of various aspects of colony’s life, the ApiPop, was created by the Italian research team [68]. Due to the user-friendly interface and high modularity of the considered aspects, authors propose to use it for educational purposes – the relative simplicity of implementing modifications and not requiring expertise in programming is its additional advantage. The model enables the use of real-life weather data and calibrates it according to the colony development observational data. It is additionally equipped with a module that calculates cell availability.

A similar approach is presented by the Bee++ model [69], using C++ language. Its authors claim that it can be easily modified by the user once it is freely downloaded from the website. However, these claims were not confirmed as the declared website address is not accessible.

The most well-known model representing the above-mentioned highly modular and intuitive modeling approach is the BEEHAVE [70]. It is the most complex, freely available model nowadays, describing the development dynamics of the whole colony combined with varroa viruses and the foraging model. Foraging processes are simulated in a time scale of minutes, while the rest of the processes are based on a 1-day simulation step. Considered parameters are external stressors (*Varroa* mites, viruses, pests), the foraging model, and the sex and age composition of a colony. The BEEHAVE partially implements also the HoPoMo model [71] to simulate the bell-shaped provision of nectar and pollen on a food patch. The main aim of the BEEHAVE is to simulate honey bee colony response to various stressors to provide beekeepers, researchers, and regulatory entities with a tool enabling them to define the levels and combinations of stressors putting bee colonies in direct danger. The BEEHAVE is described by the number of researchers in numerous publications [72], [73], [74], [75], [76], [77]. The European Food Safety Authority uses it to model the impact of various stressors on honey bee colonies [37].

The aforementioned HoPoMo model [71] describes honey bee colony dynamics and resource management. It considers food quality’s impact on the colony’s survival potential, such as availability of pollen, brood cannibalism as a protein source, and resource availability. Yet, it does not include in-hive water management, thermoregulation, or queen-supersedure process.

Another well-known and broadly used model is the one proposed by Khoury et al. [78]. A large number of papers describe models developed on the basis of the model, with minor changes in its parameters [39], [59], [79], [80], [81], [82], [83]. It focuses mostly on population dynamics and adult bees’ death rate impact on the colony collapse. However, the basic version of the model [78] is said not to be applicable to colony collapse disorder (CCD). It presents a highly specific approach to the colony demographics and the dependence between the number of in-hive bees and the number of brood. The model enables the establishment of colony seasonal/annual growth cycles.

A model combining the HoPoMo [71] with the Khoury [78] also considers pheromones' impact on the maturation of worker bees as well as the survival rate reduction during low food availability seasons [84].

The Virtual Bee Hive (VBH) is one of the models that significantly differs from the rest of the existing ones. It was proposed as preliminary work by Gallo and Witkowski [85] and simulates colony life and structure by simulating individual specimens and interactions between each of them. It is based on C# language and uses Unity 3d for visualization. VBH simulates the dynamics of a whole bee colony and its major functions in a basic way. It includes male specimens, comb maintenance, foraging, temperature regulation, and hive thermal models. Uniquely, it offers a visual presentation of all individual specimens included in the model.

Another example of modeling whole colony behavior through the behavior of individual bees is the ApisRAM, an agent-based model in which each bee is also modeled as an individual agent [86]. The key feature of this particular model is to represent bee health. The behavior of the colony, similar to the VBH, is a result of decisions made by individual specimens.

3.2.3. Category II: Partial models

This category of models describes one highly specific aspect of colony life. Some of the available models focus on the nest-site selection process [87], [88]. In most cases, models focusing on one specific colony activity do not consider drones, as they are not directly involved in the execution of related tasks. A very interesting example of a highly specific model is BEEAMATE, created as a sub-model for the aforementioned BEEPOP and simulating the genetic composition of a colony [66].

A separate group of "partial" models is composed of those describing colony failure probability caused by environmental pesticide contamination after leaving the hive [41], [89]. Due to their scope, only forager bees are considered. Attempts are being made to use them as a linkage between environmental pesticide contamination and colony collapse disorder [89].

Rumkee et al. [90] proposed the model to assess the relation between food-related behavior, pesticide concentration and exposition of specimens above some theoretical threshold. It can be used as a part of a bigger model, such as the BEEHAVE [73], for assessment of the required complexity of the realistic in-hive pesticide exposure and, therefore, improving its simulations.

A separate category of partial models of bee colony is composed of differential and integral equation-based mathematical models. In most cases, they focus on a very specific scenario, such as *Varroa destructor* impact on honey bee colony [38], or determining the apparent longevity of the colony based on the limited data [91], to improve a general understanding of the problem. Due to the very specific scope, such models consider only the aspects directly involved in the research process.

Very few of the available models consider drones. A few available solutions enable using real-life observational data as input for modeling. The number of models enabling linking the colony's strength with its pollination potential is very limited.

4. Biological data collecting

Due to the very limited knowledge regarding any insect pollinators' ability to survive space travel, in order to create a model reflecting the actual impact of g-force on bee colony development, it was necessary to conduct a suitable experiment to collect necessary data. Honey bee specimens were initially exposed to hypergravity in a rocket launch simulator, followed by observation of reproduction behavior in the apiary in the experimental beehives. The chapter describes the methodology of collecting and initially analyzing the data used for the model's creation.

4.1. The experiment

4.1.1. Methodology

4.1.1.1. Specimens' selection

The carniolan honey bee (*Apis mellifera carnica*) of the Galicja line was used for the experiment. Eight seven-day-old queens were artificially inseminated with the semen of the drones originating from a single queen of the same line, but different colonies were acquired from the specialistic apiary (Pasieka Szeligów, Wielkie Drogi, Poland). One queen was inseminated with the semen of six drones and placed in a queen mailing cage along with 25 nursing worker bees right after the insemination. After several hours, the so-prepared queen was transferred to the mini mating box with a closed exit to prevent it from performing mating flights.

Experimental and control groups were established, each composed of four artificially inseminated queens, accompanied by a cohort (9–14) of young bees (attendants). The presence of attendants during the experiments reflected the bees' natural environment.

4.1.1.2. Compliance with Ethical Standards

According to the decision of the 2nd Local Institutional Animal Care and Use Committee in Krakow, no approval from the commission was needed for the experiment. All experiments were carried out according to the ARRIVE guidelines [92].

4.1.1.3. Hypergravity

Hypergravity conditions were simulated by the Human Training Centrifuge (HTC), owned by the Military Institute of Aviation Medicine in Warsaw, Poland (Fig. 1). HTC enables simulation of every known acceleration pattern with values ranging from −3 to +16 Gz. The device's main arm is 8 m long, and the maximum roll and pitch for angular acceleration is 8 rad/s^2.

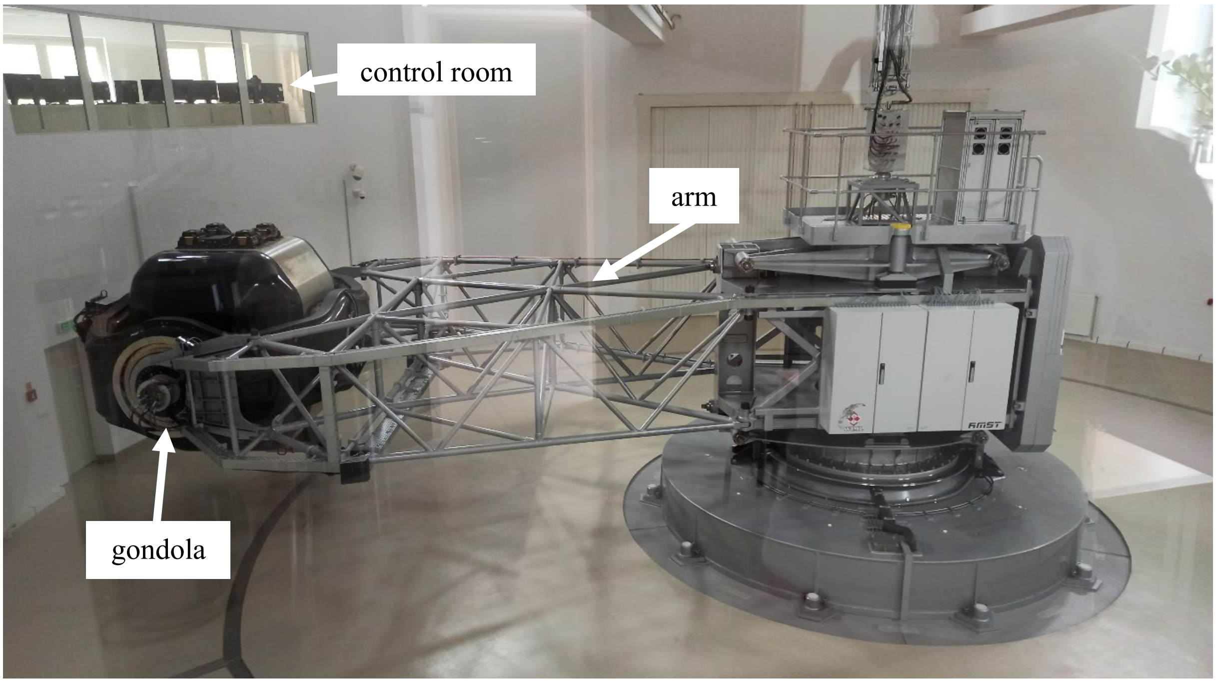


Fig. 1 Picture of the Human Training Centrifuge with the main elements marked and named. (Source: own materials)

At the time of the experiment planning, the most common rockets used for crew and cargo transportation were *Soyuz*-type. For that reason, the acceleration pattern characteristic for their launch was used, visible on Fig. 2.

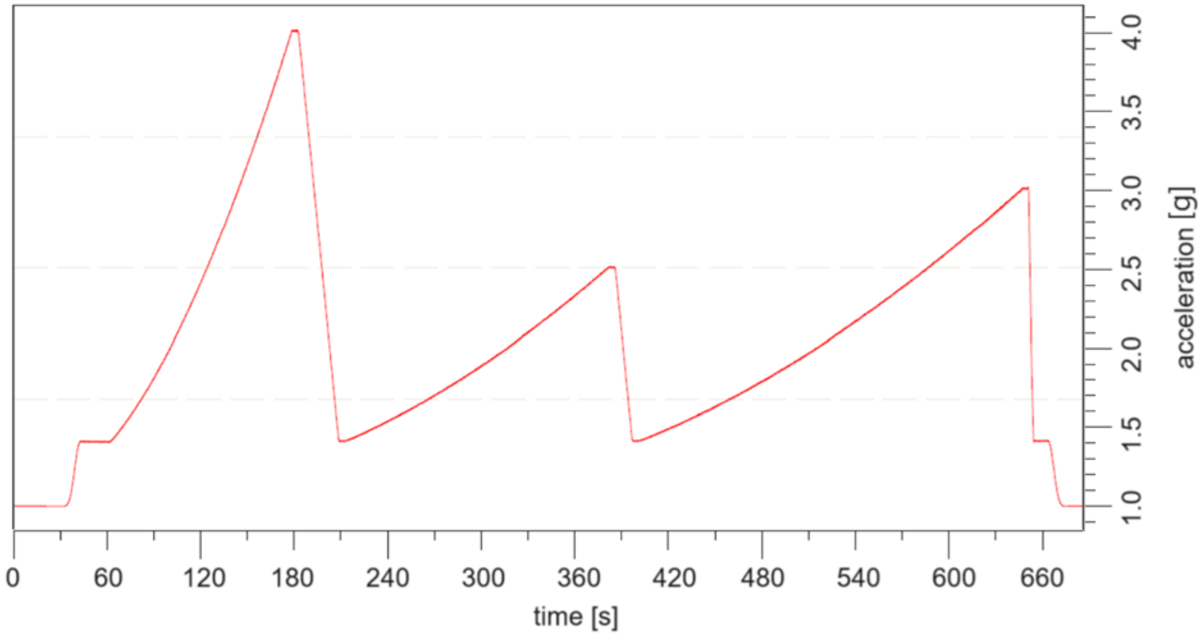


Fig. 2 Acceleration profile of the launching Soyuz-type rocket, used during the experiment (Source: Military Institute of Aviation Medicine)

During the *Soyuz* launch, three gradually increasing acceleration peaks occur: the first of 4 g in the 180th second, the second of 2.5 g in the 380th second, and the last one of 3 g in the 645th second. The increases are divided by hypergravity value drops down to 1,4 g after reaching each acceleration peak. The whole pattern execution time is 660 seconds.

4.1.1.4. Environment control device – *BeeO!Logical* payload

During the HTC experiment, both the control and test sample queens in queen mailing cages with attendants were placed in the *BeeO!Logical* devices, a rocket payload dedicated to pollinators' examination [93]. The instrument consists of three main elements: an antishock case, thermal isolation, and the internal part. Fully assembled *BeeO!Logical* has a standard size of 3U (10x10x30 cm), and its mechanical properties ensure the experiment's safe and stable conditions, such as consistent temperature or air composition. Biological sample compartments ensure good air quality for a time of no less than two hours. Its previous version was used for the preliminary research on honey bee workers' survivability of a sounding rocket flight [33].

The device has two identical copies, enabling the performance of the experiments requiring a control sample. The total time of in-payload enclosure for the test and control samples was 27 and 28 minutes, respectively.

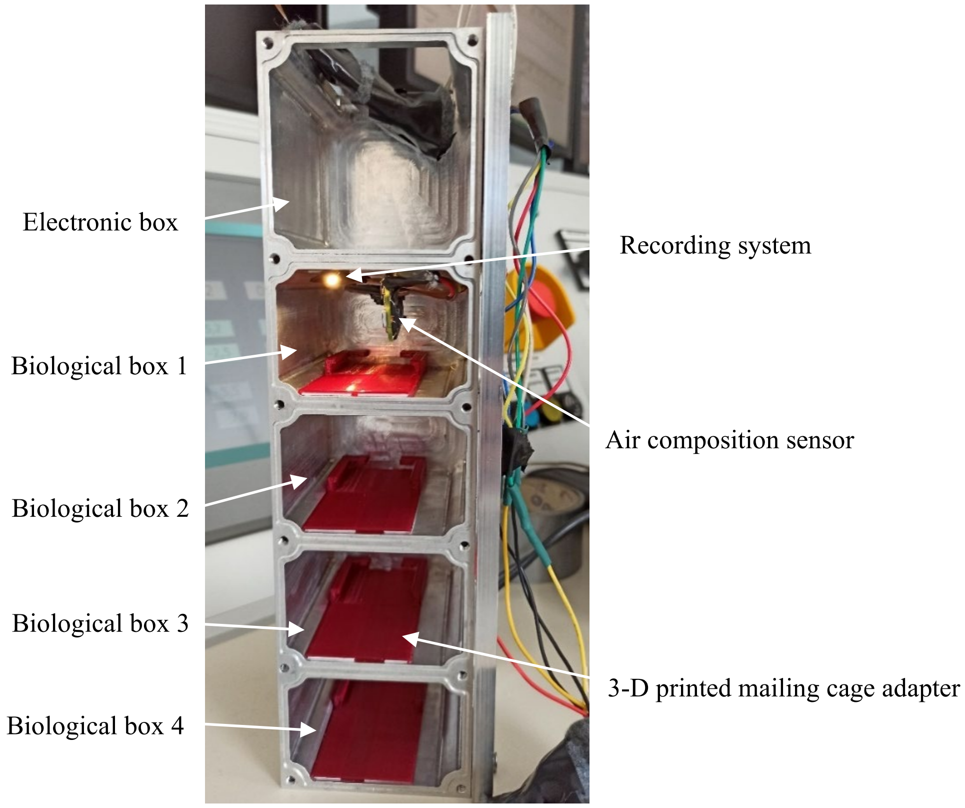


Fig. 3 Internal structure of the BeeO!Logical payload – electronic and biological compartments and specific system components are marked on the picture (Source: own materials)

Performed experiment, due to the possibility of the HTC to stabilize the temperature inside the capsule where the experiment was placed, did not require the usage of an anti-shock case and thermal isolation. Therefore, only the internal part of the payloads was used (Fig. 3).

4.1.1.5. The HTC experiment

Honey bee queens, along with attendants, as specified in Table 1, were transported from Cracow, Poland, to Warsaw, Poland, by car on June 30, 2021. On July 1, at 10:37 am, samples were placed in the *BeeO!Logical* devices were previously randomly divided into test and control samples. While the test sample was placed in the Human Training Centrifuge, the control sample was left in the control panel room. After an 11-minute-long centrifugation, both samples were recovered and opened. Examined specimens in the mailing cages were placed back in the transport box and, on the same day, transported by car back to the *Pasieka Szeligów* apiary. They were left there for initial observation and the start of laying eggs.

4.1.1.6. Setting up experimental hives

The standard and successful procedure for giving the artificially inseminated queen bee to the hive in the *Pasieka Szeligów* apiary is as follows:

- An artificially inseminated queen (AIQ) is given to the mating box.
- After 12-16 days, AIQ starts laying eggs.
- AIQ is placed in the queen mailing cage with the attendants.
- The colony, which is queenless for at least 9 days, is chosen.
- AIQ is given to the chosen colony in the queen mailing cage.
- The AIQ is released from the cage by the worker bees after 24–72 hours and starts laying eggs again.

Due to the fact that the queen is given to the hive that other queens primarily occupied, the frames may contain all bee development stages, as well as food stores. Due to the above, after transportation of the hives from the specialized apiary *Pasieka Szeligów* to the final location (experimental apiary), the contents of all hives were noted, and later changes were observed with reference to day 0 (August 3, 2021), which is defined as the day of transporting the hives to the final location.

After a month, five of the eight colonies in experimental beehives were transported to their final location. Two weeks later, on August 3, 2021, another queen was transported along with additional worker honey bee packages for the enlargement procedure required for the successful overwintering of the colonies. The transportation date of the queen was affected by its delayed acceptance to the colony.

Two out of eight queens were not accepted in the colonies to which they were given. Therefore, it was not possible to examine them in the described analysis. As one of the rejected queens was from the test sample and the other from the control one, it was concluded that a lack of acceptance was caused by a natural factor and that the queens' exposition to hypergravity did not affect their acceptance probability.

4.1.1.7. Data Acquisition Procedure

Data was acquired from August 4, 2021 (day 1) to April 29, 2022 (d. 269). Inspections were taken one to three times a week, which resulted in 12 to 15 controls per hive in 2021. After the successful overwintering, three additional inspections were performed once every two weeks. controls were conducted every other week, resulting in three inspections per hive in 2022 in total.

Mini-plus hives were chosen for the experimental hives during the fecundity and fertility analysis. Bee breeders and scientists commonly use such a hive type as it allows for natural colony development, simultaneously limiting the required work by beekeepers. The beehives were placed in the experimental apiary located at AGH University of Krakow (50°03′60″, 19°54′02″) in Poland.

The mini-plus frame has external dimensions of 215 mm (±2) per 168 mm (±2) with internal dimensions of 150 mm (±2) per 200 mm (±2), which the wax foundation can effectively occupy. The single side of the frame has from 1,127 to 1,176 cells and varies with respect to the possibility of effective use of cells on the edges, with the mean number of cells per side of the frame equals 1,152 (rounded up). This relatively constant number of cells per frame enabled recalculating the area occupied by, e.g., eggs to their exact number when needed.

The Liebefeld method was adopted for the number of brood and food (both nectar and pollen) stores assessment [94], [95] enabling rapid measurements, limiting the negative impact on colonies, and is characterized by a low variation in results [94], [96]. Instead of the coverage expressed in decimeter square, the percentage of the entire comb face was estimated with the proportion of the total to the smallest area evaluated maintained (10dm2 – 0,1dm2 vs. 100% – 1%). The method was modified due to the relatively small size of the mini-plus frame compared to the standard hives.

Each frame was inspected separately, starting with estimating the area occupied by the capped brood due to its high accuracy. Next, the area occupied by the food stores, eggs, and larvae were estimated. Each stage considered the total area to be not greater than 100%. Recalculating the area to the number of cells was possible due to the frame's fixed measurements, as described above.

Data on the number of adult bees was not collected due to their abundance being combined with multiple factors and is not exclusively limited to queens' fertility.

4.1.1.8. Beekeeping procedures detailed schedule

To lower the number of variables differing colonies development, the same beekeeping procedures were applied to all the colonies. Additionally, no honey was harvested until the end of the data collection (after day 269). All the major beekeeping actions, along with observations and comments, can be seen in Table 1.

Table 1 Beekeeping procedures schedule

Date	Experiment day	Control ("Y" - yes; "-" - no)	Feeding ("S" - yes, with sugar syrup; "I" - yes, with invert syrup; "-" - no)	Beekeeping procedures	Comments
03.08.2021	0	-	-	Hives identification	Queen no. 40 without marking number - exclusion from the experiment
04.08.2021	1	Y	-	1st full control	-
09.08.2021	6	Y	-	-	-
11.08.2021	8	Y	-	-	-
13.08.2021	10	Y	-	-	-
16.08.2021	13	Y	-	-	-
17.08.2021	14	-	-	Enlarging colonies with boxes with workers	Delivering the last hive with queen no. 39
18.08.2021	15	Y	-	Excluders removal in controlled hives	-
20.08.2021	17	Y	-	-	-
23.08.2021	20	Y	S	Applying medication strips (*Apivarol*)	-
25.08.2021	22	Y	-	-	-
26.08.2021	23	Y	-	-	-
30.08.2021	27	-	S	-	-
02.09.2021	30	Y	-	-	-
03.09.2021	31	Y	-	-	-
05.09.2021	33	Y	-	-	-
07.09.2021	35	Y	-	-	-
08.09.2021	36	Y	I	Weighting start	-
10.09.2021	38	Y	-	-	-
11.09.2021	39	Y	I	-	Weighting system montage
13.09.2021	41	Y	I	-	-
14.09.2021	42	Y	I	-	-
16.09.2021	44	-	I	-	-
20.09.2021	48	-	I	-	-
27.09.2021	55	Y	-	-	-
28.09.2021-13.02.2022	56-194	-	-	-	Overwintering
14.02.2022	195	-	-	-	1st spring flight observed
15.02.2022	196	-	-	Shrinking hives and closing ventilation	-
24.03.2022	233	Y	-	1st full inspection after winter	-
14.04.2022	254	Y	-	-	-
28.04.2022	268	-	-	-	Dead queen no. 39 found
29.04.2022	269	Y	-	Enlarging hives with boxes with wax-foundation filled frames, ventilation opening	-

4.1.1.9. Data normalization

The collected data were normalized and prepared for further analysis.

The hives' enlargement procedure performed on experiment day 14 introduced new frames into the beehives. Each frame contained a specific number of food stores and brood not originating from the experimental queens, which were added to experimental hives. Therefore, the data on the number of food stores, eggs, larvae and pupae in the colony after the procedure was corrected respectively. Food stores correction was applied to 2021 data exclusively, while brood number was corrected with respect to specific development stage mean duration [97].

Controls were performed 1 to 3 times per week. In order to provide the model with daily data, the data gaps between subsequent control days were addressed. Each gap was filled with the number of cells being calculated proportionally to the number registered during the previous and subsequent control, with the consideration of the number of days between observations.

4.1.1.10. Statistical Analysis

A correlation test was performed to check the correlation between each sample. Analyses were performed using Analysis ToolPak for Excel, version 2309, and the graphs presented in the subsequent sub-chapters were produced using the Excel Charts.

4.1.2. Results and discussion

4.1.2.1. Rejected Queens

In both groups, test and control one, one queen was not involved in the apiary data collection due to their rejection by the colonies. The queen from the control group was rejected in the mini-mating box due to the absence of egg-laying behavior. The queen from the test sample was rejected after the transfer to the experimental hive.

Another queen from the control sample was accepted into the colony with a significant delay (2 weeks after the start of data collection in the experimental apiary). In order to avoid introducing another variable to the analysis, the queen was also excluded.

One of the queens from the test sample qualified for the apiary data gathering procedure was observed to have no queen marking number and to differ in phenotypic traits from the rest. It could suggest its replacement by worker bees [98]. As a result, the data gathered from the queen was not used in the analysis nor for the model's creation, not to lower its quality and prediction precision. However, from a biological point of view, the information should be kept in mind due to the possible link between the queen's swapping and its exposure to hypergravity conditions. The hypothesis shall be verified and is a strong support for further research on the topic.

The above circumstances caused only 2 out of 4 queens from each sample to be qualified for the model's creation and analysis.

4.1.2.2. Hypergravity impact on queen's egg-laying quantities

Fecundity is the potential ability to reproduce. It was assessed based on the number of eggs laid by the queen.

Basic descriptive statistical tests for the number of eggs were performed for all experimental hives with the built-in Microsoft Excel tool for data analysis: descriptive statistics. For the test, only collected data was used, excluding data gaps. The calculated parameters can be seen in Table 2.

Table 2 Descriptive statistics for all hives based on data collected throughout the study

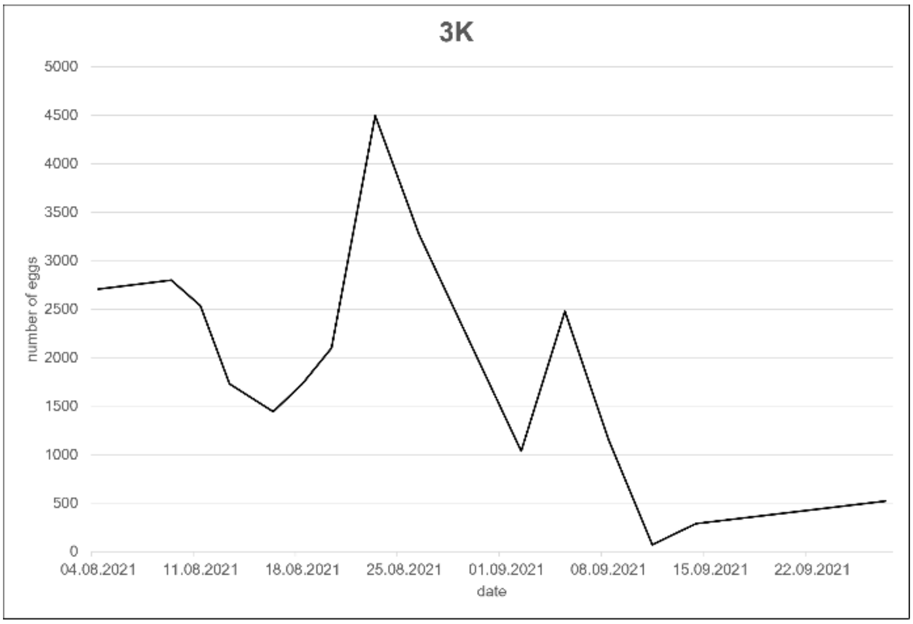
	3K	***3V***	***1D***	***1H***
Number of samples	18	17	15	16
Mean	1,931	1,375	1,586	1,608
Standard error	288	153	293	219
Median	1,855	1,440	1,336	1,590
Standard deviation	1,224	630	1,133	876
Sample variance	1,497,100	396,638	1,283,412	767,913
Minimum	69	346	202	115
Maximum	4,493	2,304	4,493	3,260
Sum	34,756	23,368	23,789	25,736
Confidence level (95.0%)	612	344	627	480

The decision to perform the descriptive statistical analysis was made apart from the seasonality of the honey bee colony development. Due to the equal hive size and the same inspection and operation procedures used for all samples throughout the observation time, colonies should develop similarly and with comparable strength. Descriptive statistics enables the comparison of colonies in terms of the overall seasonal performance.

The sample size ranged from 15 to 18 measurements in total. The mean number of cells in the hive occupied by eggs throughout the measurement season varies from 1,375 to 1,931, the greatest for queen 3K and the lowest for 3V. The mean value for the control sample is comparable for both queens, settling at 1,597 (+/-11) eggs. Similarly, the greatest and lowest sample variances were calculated for the queens of the test sample. The worst and best fecundity traits seem to have queens from the test sample, and the queens of the control sample are characterized by more stable egg-laying behavior.

The variability of the test sample in terms of the mean number of cells occupied by eggs may suggest that honey bee queens experiencing hypergravity may respond twofold: maximizing the egg production or lowering egg production, while the fecundity of queens not subjected to the hypergravity impact remains more stable throughout the whole season. This approach is supported by the historical record of research on honey bees in a space context, suggesting the negative impact of space travel on drone semen [45], [46] and the increased mortality of worker bees due to acceleration [43].

Comparing the graphs of the number of eggs laid by each queen (Fig. 4), it can be seen that the most rapid changes were observed for queen 3K. All the queens started laying more eggs after the hive enlargement procedure, probably caused by more space available for egg-laying behavior. A reduction in the number of laid eggs appeared around September 15, 2021, followed by a slight increase later, not exceeding 1,500 eggs laid in the hive 3V by the end of the season.


3K
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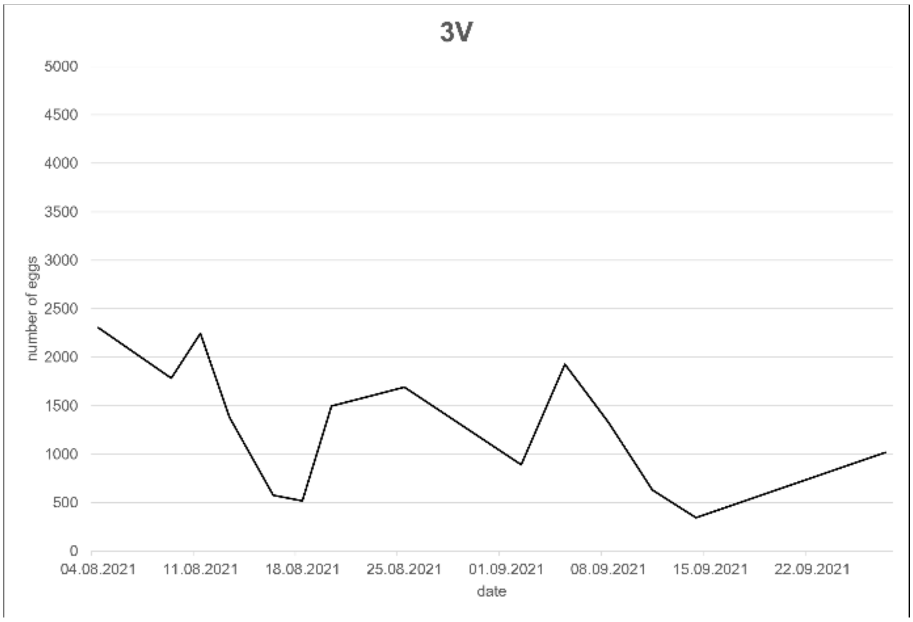
3V
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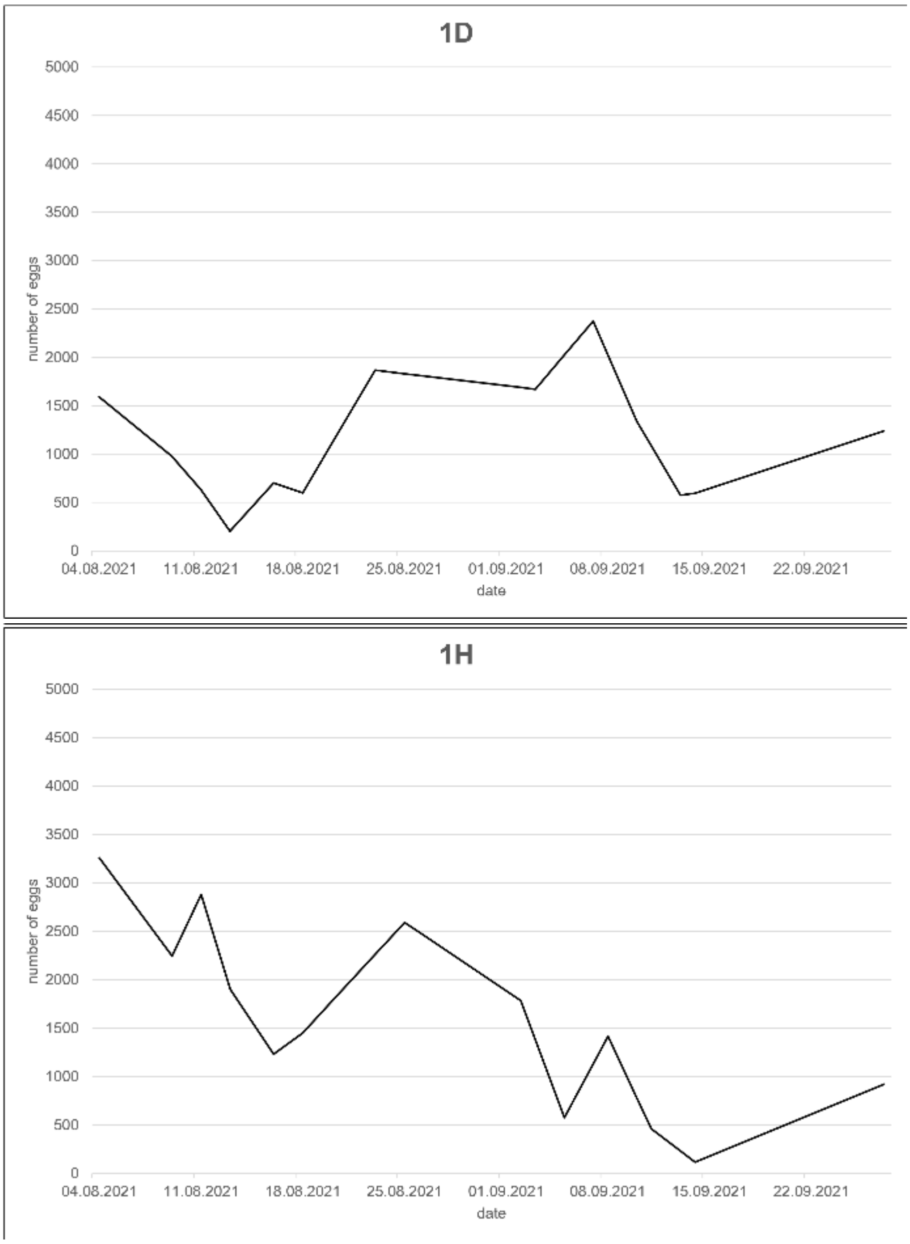
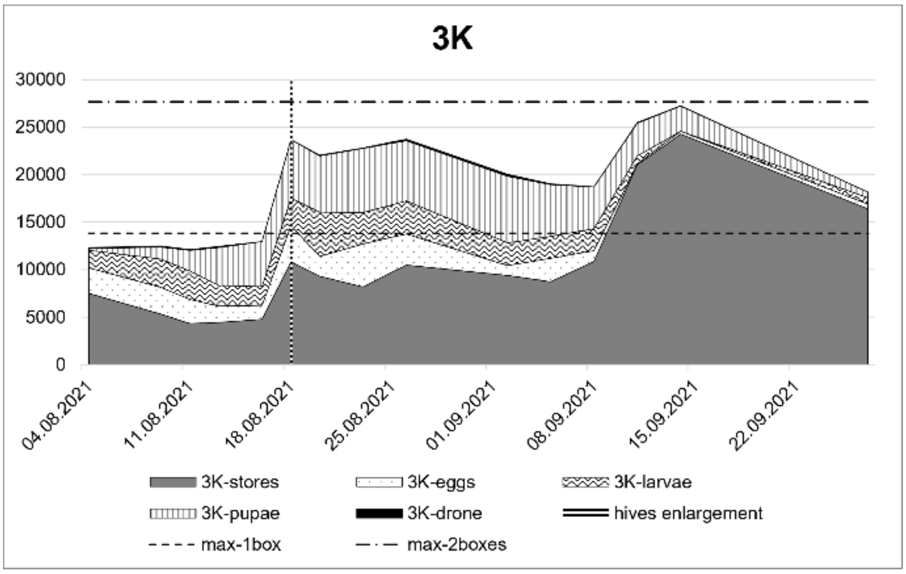


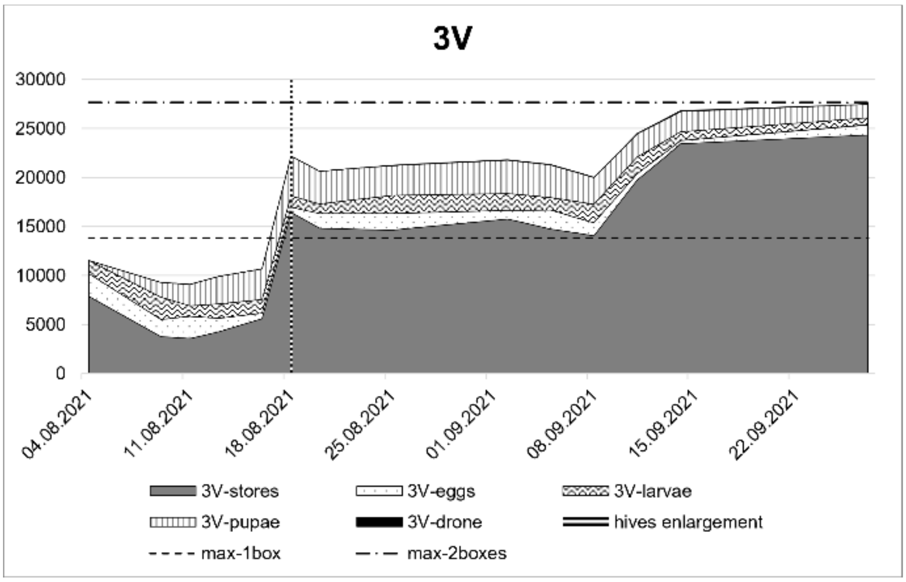
Fig. 4 Number of eggs in experimental hives throughout the 2021 season. The scale is uniform across all diagrams (Source: own materials)

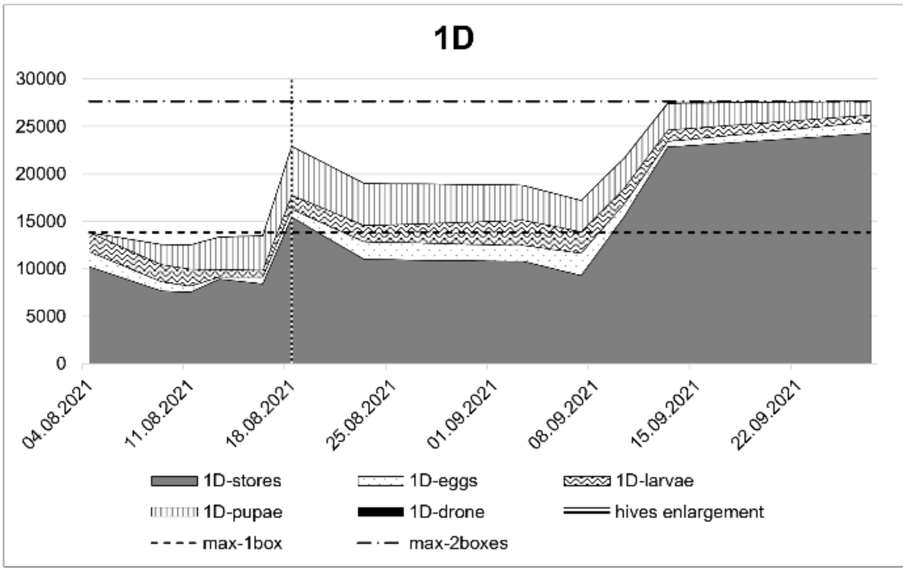
Changes in the number of laid eggs may be impacted by the queen's age [99], food type [100], and others [101]. Given parameters were uniform in the described experiment – all queens were the same age, inseminated in the same manner, given the same beekeeping procedures, and all the colonies were placed in the same area, having the same food source. For that reason, all the changes in egg-laying behavior are of experimental rather than environmental origin.

4.1.2.1. Hives composition

The general composition of the hives in 2021 is shown in Fig. 5. It can be seen that all the hives were occupied in a similar way throughout the 2021 beekeeping season, reaching the maximum volume of the hive in mid-September. Notably, there was a sudden decrease in the number of cells occupied in the 3K hive after first reaching the maximum volume capacity of the hive. The drop was mainly due to a decrease in the number of stores. The reason is unknown; however, since the 3K hive was characterized generally by the best results regarding the number of eggs laid, it could have been caused by the number of adult honey bee workers present in the hive after mid-September. The other possibility is that the drop was caused by the robbery by another colony.







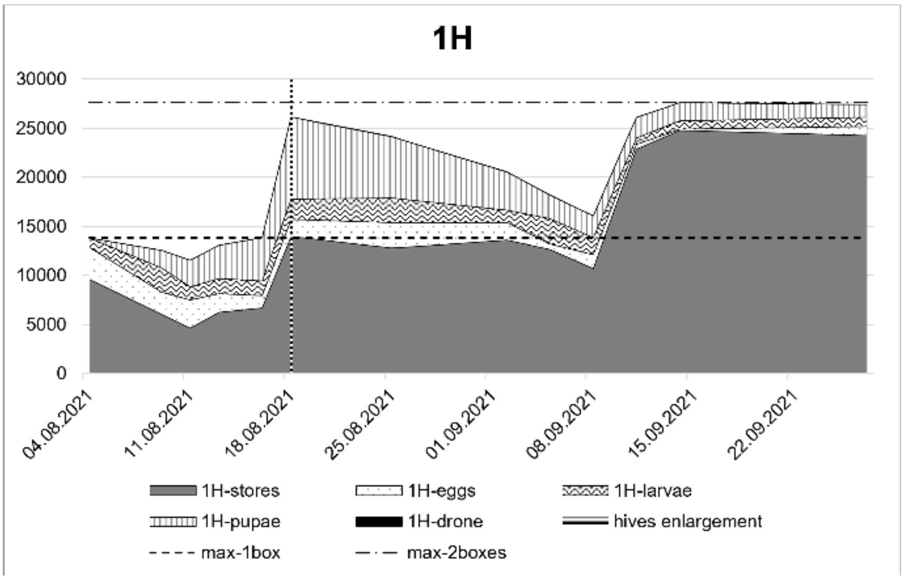
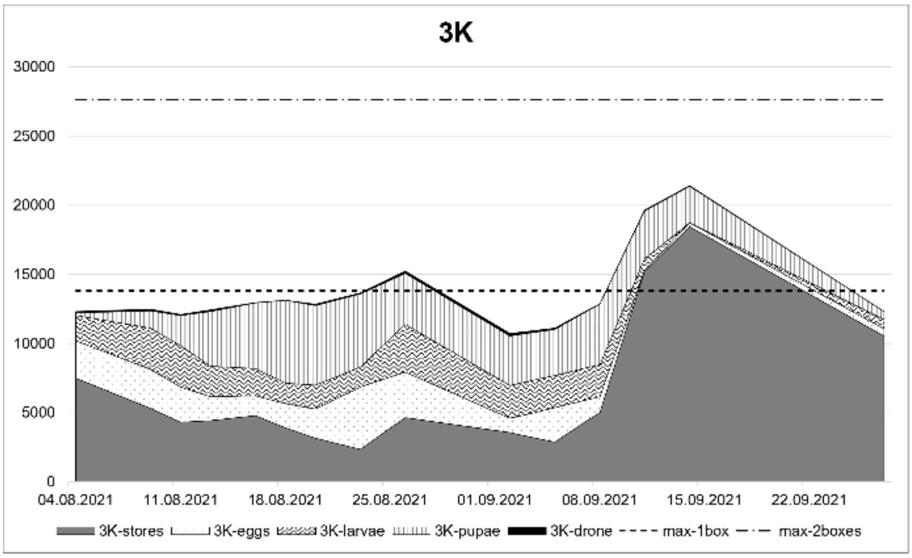


Fig. 5 Total number of cells occupied by each parameter. Similar seasonal tendencies are visible in all hives. The decrease in 3K hive volume can be seen at the end of the season; the influence of the added hive body is not considered. Horizontal dotted lines (Source: own materials)

The hive enlargement procedure caused the sudden growth on August 18 (d. 15). To each 1-body hive, another body filled with food stores, eggs, pupae, and larvae was added to increase the chances of winter survival. The additional hive bodies were acquired from the *Pasieka Szeligów* apiary. This caused an increase in the total number of cells that could have been filled (from 13,824 to 27,648 cells).

An analogous comparison of the composition of the hive was made after data correction regarding the described enlargement procedure. The total number of cells occupied was reduced by the number of cells occupied in the added hive body. 'Extra cells' were subtracted, considering bees development time. In the case of food stores, if the number of food store cells in the new hive body was greater than in the moment of adding the body, the excess number was treated as occupied by the examined queen's offspring and included in the final statistics. Taking into account the impact of the enlargement and analogous treatment of all the colonies, in terms of the amount of food stored at the end of the beekeeping season, the control sample was prepared better for overwintering than the test sample (Fig. 6).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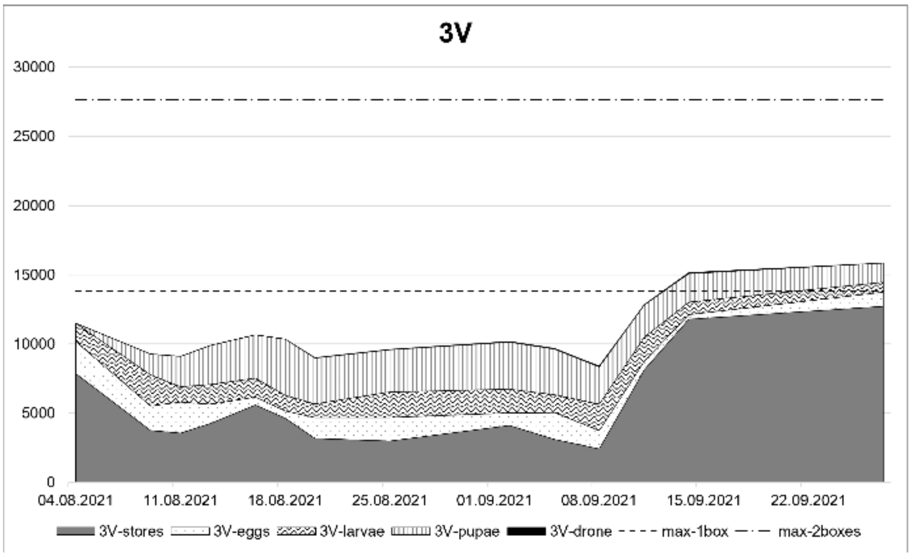
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max-2boxes

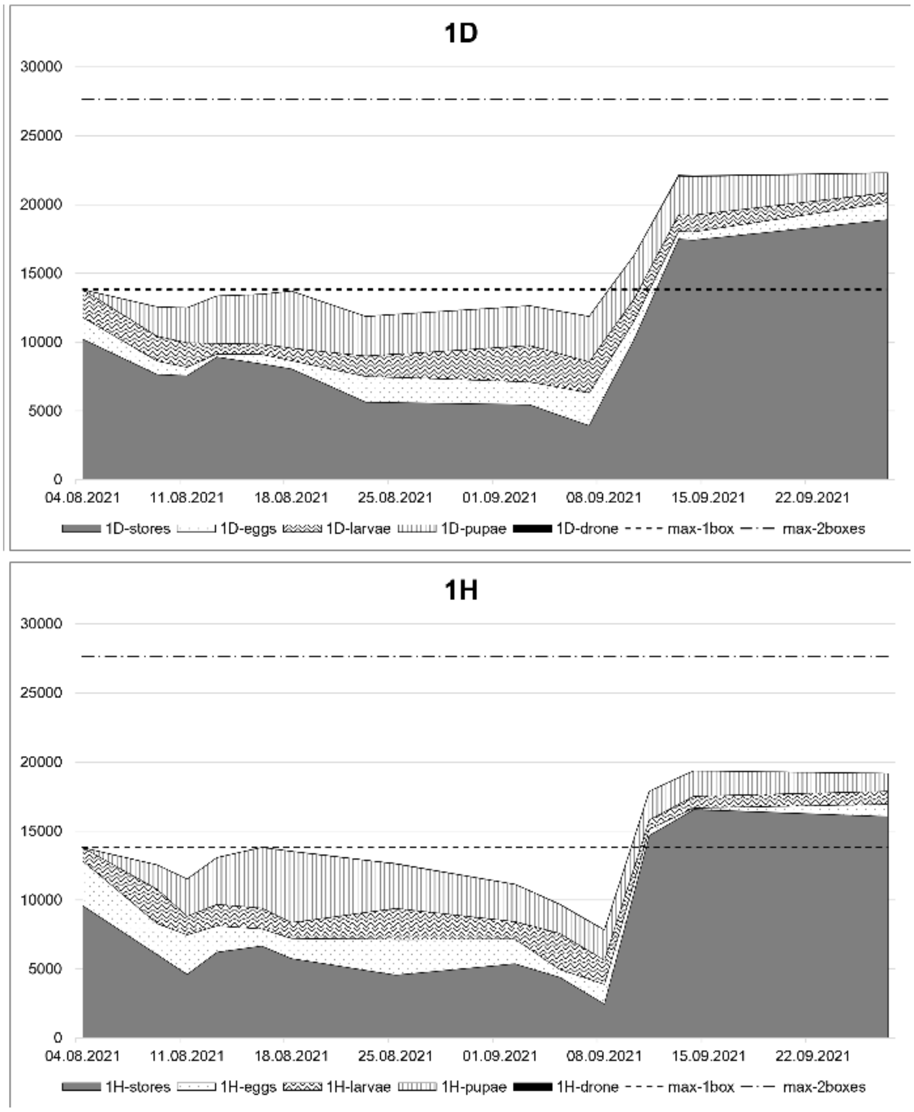
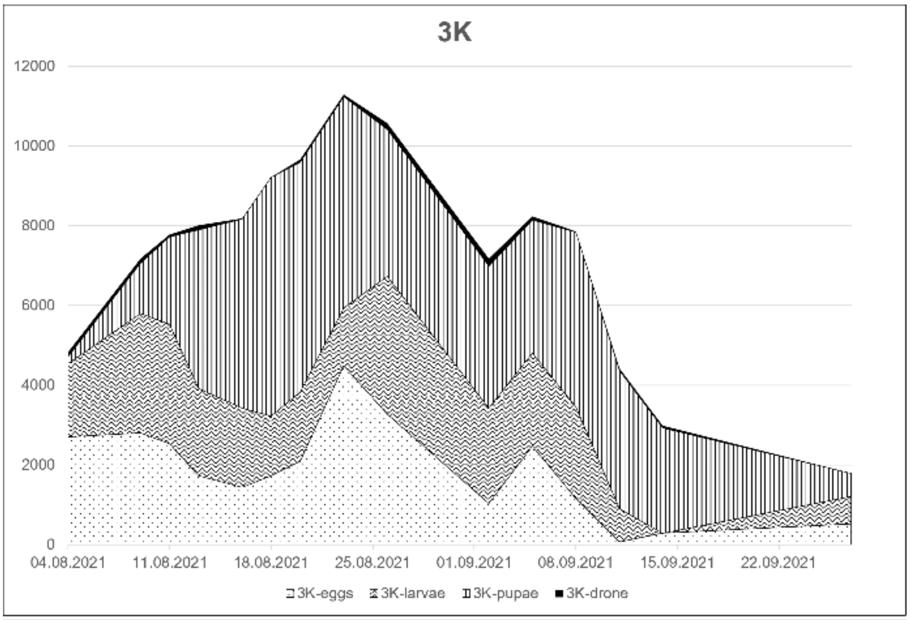
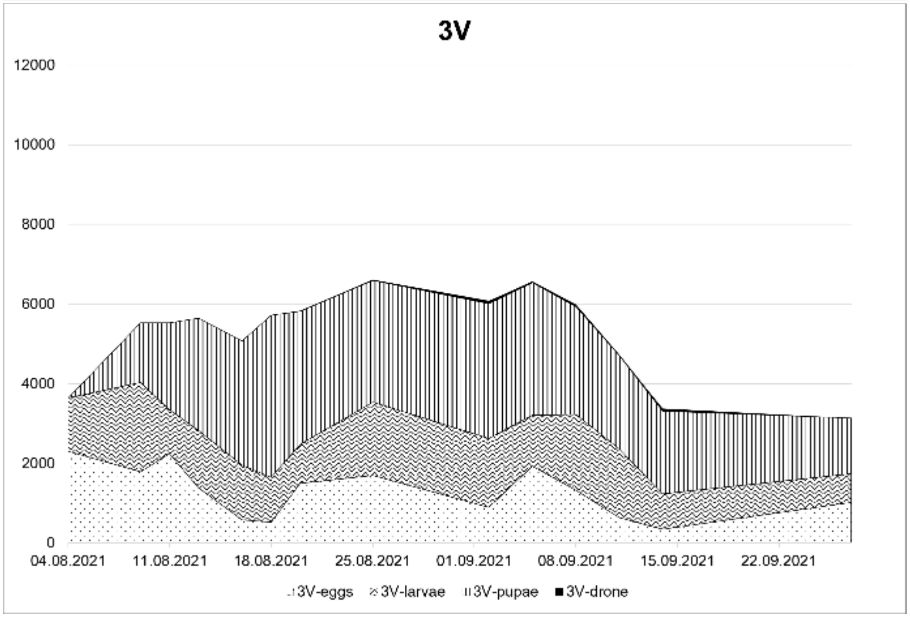
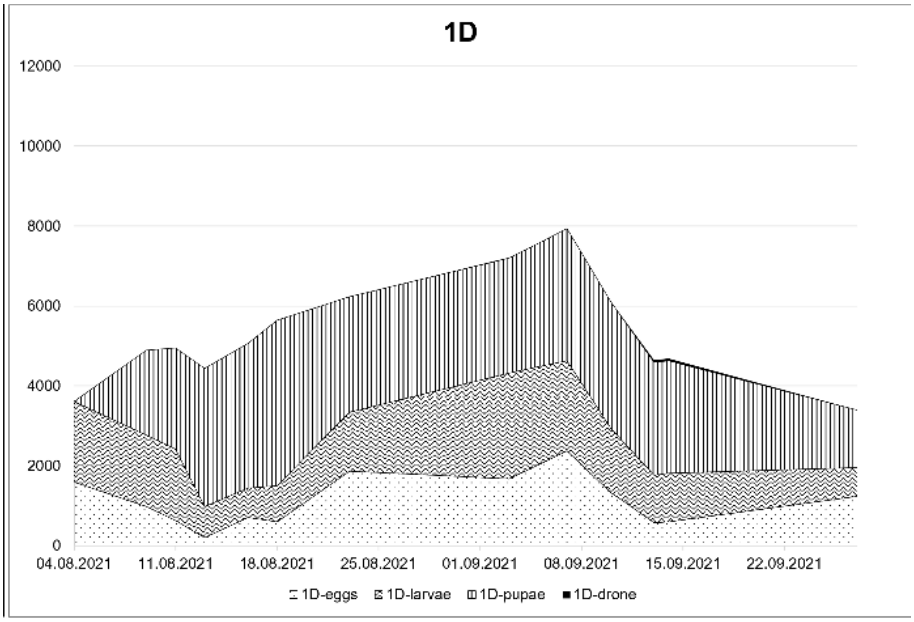


Fig. 6 Total number of cells occupied by each parameter, excluding cells occupied by brood and food stores added to the hive along with the additional hive body during the hive enlargement procedure. Horizontal dotted lines mark the maximum capacity of fully occupied hive with one and two bodies. The number of drones in a 3K hive is noticeable. The scale is identical for all graphs (Source: own materials)

Figure 7 compares the number of occupied cells between hives. Hive 3K reached over 11,000 cells occupied in total, while the other experimental sample hives had the lowest number of occupied cells from all the analyzed hives. The control hives were more consistent, reaching a maximum of approximately 8,000 occupied cells with little or no drone pupae cells.


3K
12000
10000
8000
6000
4000
2000
0
04.08.2021
11.08.2021
18.08.2021
25.08.2021
01.09.2021
08.09.2021
15.09.2021
22.09.2021
3K-eggs
3K-larvae
3K-pupae
3K-drone


3V
12000
10000
8000
6000
4000
2000
0
04.08.2021
11.08.2021
18.08.2021
25.08.2021
01.09.2021
08.09.2021
15.09.2021
22.09.2021
3V-eggs
3V-larvae
3V-pupae
3V-drone


1D
12000
10000
8000
6000
4000
2000
0
04.08.2021
11.08.2021
18.08.2021
25.08.2021
01.09.2021
08.09.2021
15.09.2021
22.09.2021
1D-eggs
1D-larvae
1D-pupae
1D-drone

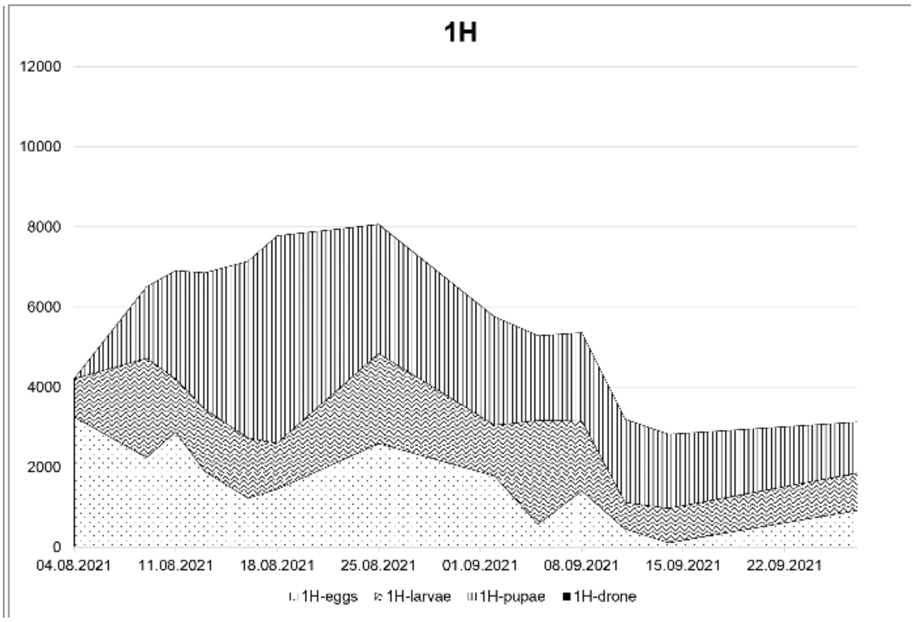


Fig. 7 Number of cells occupied by various stages of the development of bees. The maximum number of occupied cells was more than 11000 in the 3K hive, having also the greatest part of drone pupae in the composition. The scale is uniform across all diagrams (Source: own materials)

4.1.2.2. Overwinter survival and spring season start

The readiness for overwintering was assessed based on the total mass of the hive. It indicates the amount of food stored, which is crucial for winter survival. The changes in the hive mass can be seen in Fig. 8, covering exclusively the feeding period of the colonies with the invert syrup. The weight gain is similar for all hives, with no less than a 0.986 correlation. The mass of the invert left on the feeder was subtracted for the last measurement.

The observation might suggest that all the colonies entered the overwintering in similar numbers. If any colonies were overrepresented in the summer worker bees, which die out in autumn, a collapse would be visible on the graph.

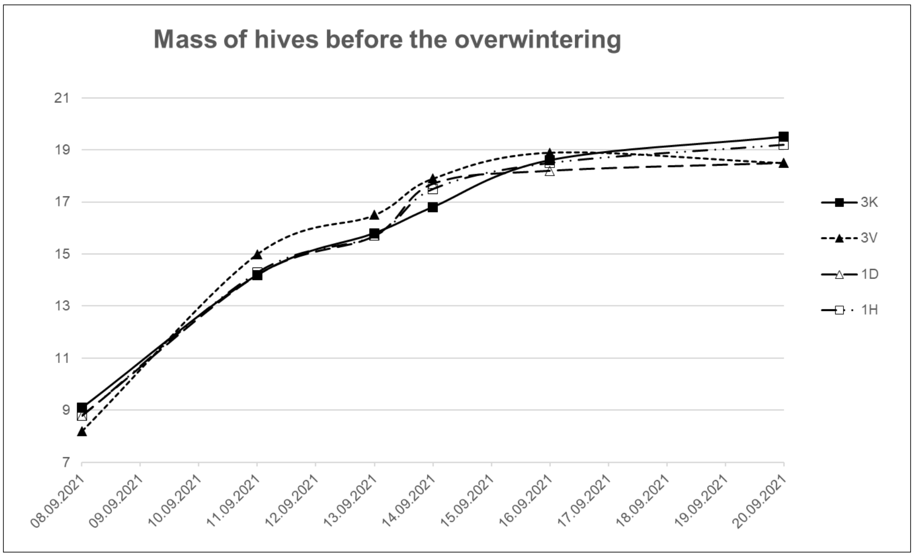


Fig. 8 Evaluation of overwinter readiness based on the hive mass: All samples reached the same mass level at the end of the beekeeping season (Source: own materials)

Another issue related to overwintering is pathogens infestation, with an emphasis on *Varroa destructor*. To limit its possible negative impact on overwintering success all colonies were treated, as previously shown in Table 1.

All examined colonies survived the winter in good condition and correctly resumed activity in spring. The number of eggs laid at the beginning of the 2022 beekeeping season can be seen in Fig. 9. In general, experimental sample colonies performed worse than the control colonies and had a greater number of drones, emphasizing hive 3K. The higher drone production might be an indicator of issues with stored semen. Typically, it is characteristic of older queens which run out of sperm [102], [103]. The early droning of experimental queens might suggest the above-mentioned or other physiological-related issues.

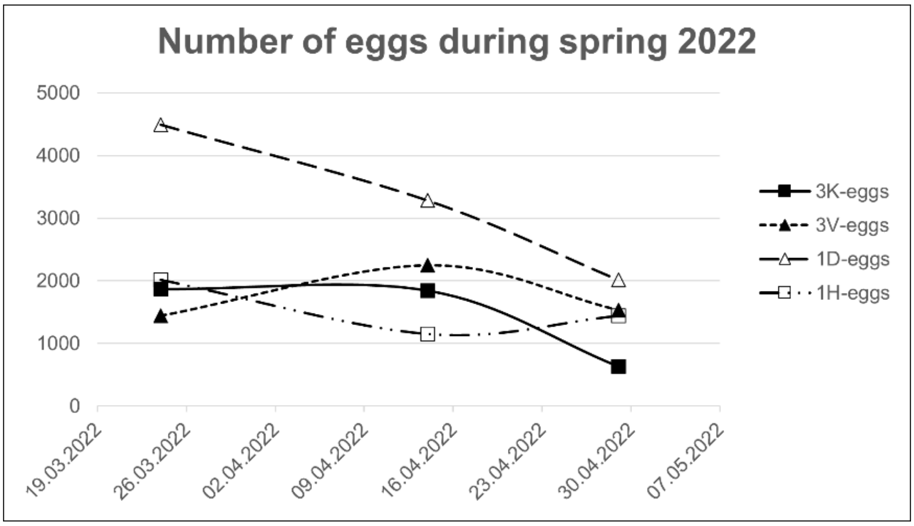


Fig. 9 Number of eggs laid at the beginning of beekeeping season 2022 – hive 1D had more than twice as many eggs as the rest of the hives during the first measurement. In all the hives during the last control, the number of eggs dropped below 2000 (Source: own materials)

Controls of the colonies were performed until the end of April 2022. Since May 2022, controls have been stopped due to swarming behavior in the examined colonies. Such an event causes a reduction in the number of eggs laid by the queen, and any action performed to mitigate the swarming in the colony could directly impact the experiment results. For that reason, a decision was made to stop gathering data. Later, queen 39 was found dead, and queen 40 swarmed. All remaining colonies and experimental queens survived until the end of the 2022 season and were overwintered again.

4.1.2.3. Data Acquisition Procedure Bias

The same person took observations each time, ensuring a stable bias for all measurements, if present. As the research on the Liebefeld method proved, the smaller size of the hive increases the precision of the estimates, and a possible variation from the real number is stable [94], [96].

4.1.3. Experiment limitations

The author is aware of the limitations of the presented experiment. The most significant limiting factor is the size of the test and control groups, preventing from drawing statistically significant conclusions for the entire species. Although implementing larger sampling would provide more significant statistical power and natural variation observed among honey bee colonies, acquired data enables starting the work on the relevant computer model of a bee colony.

Another significant limitation is the lack of spring/early summer observation. The seasonal nature of *A. mellifera* biology leads to a significant variation in development dynamics throughout the year. Young queens can be successfully inseminated (depending on the season) as late as the end of May.

Considering the time from insemination through the experiment to successfully implementing queens into the colonies, the observations could not start before June. The analogous experiment should be carried out with observation throughout the whole beekeeping season, which would allow the evaluation of the fertility of the queens throughout the year.

The fertility results and survivability of the colonies could have been biased by the hives enlargement procedure performed on August 18, 2021. Available precautions were taken to reduce bias; however, the appearance of new worker bees could have affected the functioning of the colonies. The decision on hive enlargement was made for the good of the examined subjects and for the extension of the observation time of the colonies.

The study considers only the very initial part of space travel, the rocket launch. Various acceleration patterns and g-force values should be examined for the broadening of the perspective. It is crucial due to the rapid development of space transportation systems such as those offered by SpaceX or NASA. The condition of honey bees in the context of the subsequent stages of space travel should also be of interest to the scientific community. This includes issues such as long periods of microgravity impact on the condition of the honey bee colony as well as the effectiveness of *A. mellifera* as a pollinator under lowered gravity.

5. The model

5.1. Assumptions

Due to the specific purpose for which the model will be used during its further development in the examined context, several assumptions have been made that shall be met by the chosen model. The assumptions contain several functionalities and aspects that shall be considered to enable adjustment to the research needs.

5.1.1. Colony development

As the basic goal of the model is to simulate the development of the whole honey bee colony, the chosen model' basic function shall be modeling of the bee colony development. It is necessary to establish how the colony development pattern changes depending on experiencing the hypergravity by the queen.

5.1.2. Queen traits

Due to the queen being, in normal conditions [104], the only specimen in a honey bee colony able to reproduce, meaning sustain the life of the whole colony, it was assumed that the model should enable manipulation of the queen's traits, such as age, egg-laying pattern or egg-laying rate, as a build-in or easy to implement option.

5.1.3. Weather

Honey bees, after the rocket transportation to another celestial body, will be used in a greenhouse so the weather conditions will be stable through the whole period of the colony development. However, during the observational phase of the experiment performed to gather the necessary data on the development of the honey bee colony with the queen given to the acceleration related to rocket launch, described in Chapter 4 weather conditions were not consistent throughout the whole observation period and changed in a pattern characteristic for the region (Małopolska region, Poland). Therefore, the model shall enable the provision of input data on weather conditions to simulate both experimental conditions on Earth and future stable conditions in extraterrestrial greenhouses.

5.1.4. Simulation time

Considering the average lifespan of the queen [105], uninterrupted replaceability of the colonies and the average Earth-Mars transportation time, with safety margins applied, the simulation should have the ability to continue for the time of at least two years.

The observational data from the biological experiment (Chapter 4) after implemented adjustments have a resolution of no less than one day. Regular hive inspections are also performed no more frequently than once a day, and bee development is usually described in the literature on the scale of days [97]. Therefore, the minimum simulation resolution shall be no less than one day.

5.1.5. Input data

Due to the novelty of the research area and related to this limited number of available data on the topic, the model needs to provide an option to define individual starting conditions regarding the hive composition (number of eggs, larvae, pupae and food stores) as input data. Such a solution will simplify its further development as well as usage by non-specialized personnel in the area of bee colony modeling topic.

5.2. Basic model selection

The level of complexity and time required for the development of a complete model of a bee colony led to decision of using one of the available solutions as a basis for planned work. Additionally, such a decision increases the chances of using the developed model in the future, as the audience will be familiar with its basic functions and the reliability of predictions.

Models introduced and initially compared in Chapter 3.2 were additionally investigated and analyzed, with respect to the assumptions made in Chapter 5.1 in order to establish the model which will be used during further works. They were compared in terms of the considered factors. The comparison of the models' various features is presented in Table 3.

Table 3 A comparison of available models of a honey bee colony development.

Factors' group	Factor	BEEHAVE	BEEPOP	BeePop+	Bee++	ApiPop	Migacz & Tadeuisiewicz	VBH	ApisRAM	Khoury	HoPoMo
Model basic features	Time step	1d[a,b]/ 1min[c]	1d	1d	1 min	1d	1d	N/A	10 min	1d	1d
	Sensitivity/stability analysis	+	+	+	+	-	-	-	-	+	+
Sex/cast	In-hive bees	+	+	+	+	+[d]	+	+	+	+	+
	Forager bees	+	+	+	+	+[d]	+	+	+	+	+
	Male bees (drones)	+	+	-	+	+	-	-	+	-	-
	Predicted life span / bees age	+	+	+	+	+	+	+	+	+	+
Pests etc.	Varroa mites	+	+	+	+	+	-	-	+	-	-
	Viruses	+	+	+	+	+	-	-	+	+	-
	Pesticides	+	+	+	+	+	-	-	+	-	-
	Food contamination	+	-	+	+	-	-	-	+	-	-
External conditions	Landscape input data	+	-	-	+	+	-	+	+	-	-
	Seasonality	+	+	+	+	-	+	-	+	+	+
	Weather input data	+	+	+	+	+	+	-	+	+	+
	Wintertime	+	+	+	+	-	-	-	+	+	+
In-hive features	Pheromones	-	-	-	-	-	-	-	-	-	+
	Honeycomb building	-	-	-	-	+	+	+	+	-	+
	Thermoregulation	-	-	-	-	-	-	+	+	-	-
Beekeeping	Artificial feeding	+	-	+	+	+	+	-	+	+	-
	Disease/-s treatment	+	-	+	+	+	-	-	+	+	+
Queen trait	Queen age	+	+	+	+	+	-	-	+	-	+
	Queen egg-laying rate/profile	+	+	+	+	+	+	-	+	+	+
	Spermatozoa amount acquired by a queen	-	+	-	-	+	-	-	-	-	-
Colony reproductio	Swarming	+	+	-	-	+	-	-	-	-	+
	New queen production	-	+	-	-	-	-	+	+	-	-
	Drones' mating process	-	-	-	+	-	-	-	-	-	-
Food manageme	Foraging	+	-	+	+	+	+	+	+	+	+
	Food stores	+	+	+	+	+	+	+	+	+	+
	Stores type	+	+	+	+	+	+	+	+	+	+

---

[a] For BEEHAVE

[b] Except foraging processes, operating on timescale of minutes

[c] For Bee++

[d] worker bees can change their activities between house and foraging

As shown in Table 3, there are three main groups of available models, fulfilling the assumptions presented in Chapter 5.1: BEEHAVE, Bee++, and ApisRAM. They consider all the most important factors from the point of view of simulating the space colony with a queen bee which experienced hypergravity related to the rocket launch.

From the three, the ApisRAM had no sensitivity or stability analysis performed. Such an analysis is an additional asset of the BEEHAVE and Bee++ models as it provides extra evidence of the reliability of the model results and guidance in their correct interpretation. The possibility of the swarming event is considered only by the BEEHAVE model. In the given context, such a functionality might appear as crucial, as a colony after a swarming event is temporarily weaker. What is more important, the queen experiencing the hypergravity impact would be the one leaving the original hive. The appearance of the new queen after the rocket flight is an interesting combination, and possibility to explore the potential effects with the developed model was considered as a nice-to-have feature of the potential model.

What is of equal great importance is the continuity of the further development and analyses performed with the existing model. The last article describing the Bee++ model is dated for 2017, while the number of publications and reports describing the usage of the BEEHAVE or related its parts is over 30, and almost each year since its first publication, new analyses are being published. For that reason, the BEEHAVE was finally chosen as a basis for further development in the examined context.

The chosen model's additional advantage is its predictions verification by the United Nations organ, the European Food Safety Authority, for the verification of pesticide impact on honey bee colony development. It increases the model's general reliability and ensures its basic maintenance for the near future, which is not guaranteed in case the models are not supported by official European institutions. Finally, as the model is well recognized and known in the field of bee colony development simulations, chances for its usage in the future additionally increase.

5.3. Problem formulation

The BEEHAVE is a mechanistic model of a honey bee colony, which represents in-hive colony dynamics, mite infestations and foraging. It has been identified as a potentially valuable tool for predicting the development of a bee colony in which queen was given to under hypergravity conditions. However, the reproductive response of the queen exposed to hypergravity is not implemented in the existing model.

With the observed colony development changes, the following sections demonstrate how the effects of space travel can be implemented explicitly in BEEHAVE. The main focus includes changes in egg-laying rate to assess potential colony-level effects of the queen's exposure to hypergravity, but factors such as the maximum size of the beehive used, and local weather conditions are additionally considered.

5.4. Model description

A description of the basic version of the model, following the standardized ODD (Overview, Design, and Details) protocol, is available on the BEEHAVE's website (http://beehave-model.net).

For the problem considered, the BEEHAVE was additionally equipped with the parameters such as *QueenSpaceTraveled*, *SpaceTravelType*, *SpaceResponse*, *SpaceTravelDroningFactor*, *SpaceTravelDroningShift*. Moreover, the interface was equipped with additional windows for monitoring (*NumberOfCells*, *max-honey-mass*). A comprehensive list of additions and changes to the BEEHAVE model are provided in the following subsections. The changes were applied to the BEEHAVE version "BEEHAVE_BeeMapp2016".

NetLogo model: BEEHAVE_BeeMapp2016_rockettransport.nlogo requires NetLogo 5 to be opened and run (https://ccl.northwestern.edu/netlogo/); BEEHAVE is currently not compatible with later versions of NetLogo (version 6). All the simulations were based on version 5.3.1.

5.5. Summary of the BEEHAVE model adjustments

5.5.1. Simulation starting conditions

In the model, part of the alterations proposed by Agatz et al. [106] were used. Changes included switches and diagram addition to the model's interface. It allowed starting a model run on chosen day of the year and specifying the initial colony conditions regarding a number of breed and food stores. Figure 10 presents the interface part with the new input window for the simulation starting day ("exp_StartDay", expressed as a number of days from 1 to 365 range; no leap years option is considered. Default option is "0"). "exp_X-Days" input window indicates the number of days that simulation shall run, with the possibility to use pre-coded option for one day, month (30 days) or year (365 days).


Julian Day: 0
Date & Year: 31 December 1
exp_StartDay: 0
exp_X-Days: 365

Fig. 10 Simulation duration interface part, enabling providing the required period to be modelled. (Source: BEEHAVE model interface)

The alterations are of particular importance for experimental colonies, where initial conditions are monitored, and the starting day of the observation might vary from the starting day for regular colonies. What might be of even greater importance, is that it enables simulation in the required season. This will enable the simulation of controlled greenhouse conditions in the future, excluding the need to consider bees overwintering during prolonged stays.

5.5.2. Module I – queen bee assets module

To simulate the impact of space travel on queen bee assets, additional comments were added to the *NewEggsProc* code section, along with two new globals and two new input windows (switch type). Fig. 11 presents the input windows enabling an indication of experiencing the space travel conditions along with the expected response of the queen bee.

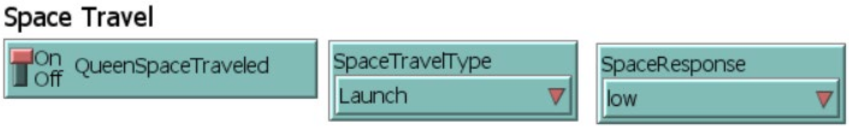

Space Travel
QueenSpaceTraveled: Off
SpaceTravelType: Launch
SpaceResponse: low

Fig. 11 Input windows visible in the model interface, enabling indication of experiencing space travel by the queen (Source: BEEHAVE model modified interface)

Code alteration introduced new parameters: *SpaceTravelDroningFactor* and *SpaceTravelDroningShift*, defined as separate global variables, set to equal 7 and -20 (numbers concluded on the basis of available field data), *SpaceTravelType*, defining which part or type of the travel honey bee queen or whole colony experienced, *SpaceResponse*, indicating which kind of the colony development response we want to simulate, and parameters of egg laying rate change in case of experiencing by the queen bee hypergravity effects, specified separately for the *high* and *low* response scenario. Numbers were calculated and concluded on the basis of field data analysis. *SpaceTravelType* was defined in the model despite the fact of having the data enabling parametrization and definition of only one type of travel (*Soyuz* rocket launch conditions experienced only by the queen and several attendants). The decision was made for simplification of further development of the model once more relevant data will be available.

The initial conditions for the model of the colony with queen bee given to the launch-related hypergravity impact are verified by the commands:

```
if QueenSpaceTraveled = true

if SpaceTravelType = "Launch"
```

If condition for space travel type choosing is not fulfilled (= “None”) the user message “*Define SpaceTravelType!”* is printed.

After verification of the initial conditions, the alteration to the worker egg laying rate is introduced as followed:

```
if QueenSpaceTraveled = true and SpaceTravelType = "Launch" and SpaceResponse = "high"

[

let potentialEggs ((MAX_EGG_LAYING * 1.31)

+ (-0.043 * (ELRt ^ 0.5))

+ (0.29 * ELRt ))

set NewWorkerEggs round (NewWorkerEggs * (potentialEggs / MAX_EGG_LAYING))


let SpaceDroningHigh (SpaceTravelDroningFactor * 0.7)

set NewDroneEggs floor(NewWorkerEggs * DRONE_EGGS_PROPORTION * SpaceDroningHigh)

set DRONE_EGGLAYING_START (DRONE_EGGLAYING_START + SpaceTravelDroningShift )

]


if QueenSpaceTraveled = true and SpaceTravelType = "Launch" and SpaceResponse = "low"

[

let potentialEggs (MAX_EGG_LAYING

+ (-0.29 * ELRt)

+ (0.14 * (ELRt ^ 0.65)))

set NewWorkerEggs round (NewWorkerEggs * (potentialEggs / MAX_EGG_LAYING))


let SpaceDroningLow (SpaceTravelDroningFactor * 0.2)

set NewDroneEggs floor (NewWorkerEggs * DRONE_EGGS_PROPORTION * SpaceDroningLow)

set DRONE_EGGLAYING_START (DRONE_EGGLAYING_START)
```

]

SpaceResponse input window, visible on Fig. 11, enables to define the expected response of the queen bee for the space travel conditions. As the up-to-date observations indicate, honey bee queens, which experienced rocket launch-related acceleration patterns, may respond two-fold – limiting the number of eggs laid or maximizing it. The chooser option enables the simulation of both scenarios separately, as available data does not enable the drawing of conclusions on the probability of the specific response type. Once such data is available, the input window will be eligible to be replaced by the pre-coded probability of a certain type of response.

5.5.3. Module II – real-life input data

The original BEEHAVE code was modified in order to enable the implementation of real-life data gathered during inspections. Modification alters simulated numbers of eggs, larvae, pupae, and drone pupae to match the ones noted during the inspections.

The altered module does not consider the number of adult bees. Queen's presence check was left in an unaltered manner. The measurement data reading was corrected to enable the module usage for observations not starting on January 1st.

Alteration also considers the food stores available in the hive. Due to the data format on the food stores available from the field study, two assumptions were made based on the literature and experimental data:

Mean honey to pollen ratio in the hive is 0.81:0.19 [107], [108]

One cell filled with pollen weights 0.23 g, and one cell filled with honey weights 0.5 g [71]

This resulted in changing the code fragment describing honey stores correction in accordance with real honey stores from:

```
set HoneyEnergyStore ENERGY_HONEY_per_g * 1000 * item 7 nextBeeMappCorrectionList
```

where *item 7* described honey stores expressed in kilograms, and *1000* recalculated the number to the required unit,

to:

```
set HoneyEnergyStore ENERGY_HONEY_per_g * 0.81 * 0.5 * item 7 nextBeeMappCorrectionList
```

where *item 7* describes honey stores expressed in a number of cells, 0.81 stands for the percentage of cells statistically filled with honey, and *0.5* represents the mean weight of a single cell filled with honey.

In the BEEHAVE interface, additional choices were added to the BeeMapp_FILE chooser: "Assessments_real_1H.txt" specifically for the data from the preliminary experiment performed, namely the data relating to the control hive 1H, and "Assessments-test.txt", being more general file chooser, designed to be used for input data file from any colony or experiment performed in the past or in the future.

The input file had to be prepared accordingly. As for the other input files used by the BEEHAVE, the input file needs to be saved in .txt file format, named in accordance with the options from the BeeMapp_FILE chooser. The recommended name, as explained above, is "Assessments-test.txt". The input file should be composed of eight columns, respectively: *date, timestep, queen?, Neggs, Nlarvae, Npupae, Ndronepupae, Nstores.*

date shall be provided in "dd.mm.yyy" format.

timestep is the order number of a day in a year, not including leap years.

queen? refers to the presence (value in the column equal 1) or absence (value = 0) of the queen.

All the other columns contain the number of cells occupied by the specific bee development stage or number of cells occupied by nectar (honey) and pollen.

5.5.4. Module III – hive type modification

Enables to change the hive type. The least important parameter, however, is worth inclusion, especially for the smallest/biggest hives models. Additionally, the amount of space available in the hive has a considerable impact on the colony development dynamics and might even lead to the unexpected limiting of the queen in the number of eggs laid.

Several monitor and input windows were added to the model, to introduce a dependency between the used hive type and a maximum number of available cells to be used (Fig. 12). Additionally, a mini-plus hive type was added to the Hive Type chooser and mini-plus frame to the Frame Type chooser. "show cells per frame" button reports the number of cells on one frame in a chosen hive-frame type configuration. The reported number is an input for the *CellsFrame* input window. The *NoOfFrames* input window requires the introduction of the maximum number of frames used in the hive during the season. A total number of cells in the hive is reported in two locations: the *NumberOfCells* monitor window located in the "Calculator: # cells" model section and the *MAX_BROODCELLS* report window in the "Colony stores" section. The number is then used as an input for the *MAX_BROODCELLS* input window, where it limits the maximum number of cells that can be occupied by all the brood stages and food stores in the hive.

HiveType: mini-plus
FrameType: mini-plus frame
NoOfFrames: 24
CellsFrame: 2300
NumberOfCells: 55200
Max-honey-mass: 27.6

MAX_BROODCELLS: 55200
MAX_HONEY_STORE_kg: 27.6
MAX_BROODCELLS: 55200
Max-honey-mass: 27.6

Fig. 12 Two sections of the BEEHAVE interface modified with the new hive type addition, enabling monitoring the maximum number of available cells and maximum mass of honey stored. (Source: BEEHAVE model modified interface)

For now, the model does not enable to mix frame types used nor changing the number of frames during the season. Adding such an option could improve the prediction abilities of the model.

6. Model behavior analysis

6.1. Verification of the code

The code was thoroughly checked during the model development. Similar to the original BEEHAVE, visual testing using the plots on the interface was performed to monitor the model behavior, most often by tracing the colony development with the colony structure workers and egg-laying plots. User commands are defined to be displayed to the user in case of insufficient information on starting conditions.

6.2. Added modules performance

6.2.1. Basic model version and default conditions

If no space conditions are defined with the input windows visible in the interface, the model produces the same dynamics as the BEEHAVE version it was based on (BEEHAVE_BeeMapp2016).

Default beekeeping modeling scenarios with minor changes were used. Specific values of the parameters considered in all the following subsections are defined in the model interface as shown in Table 4, unless indicated otherwise.

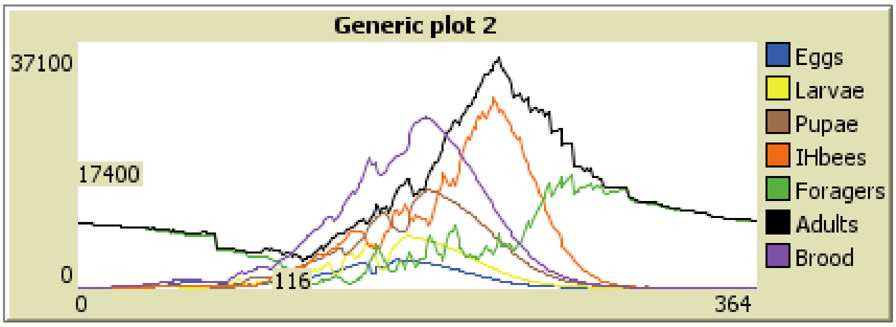
Table 4 List of default values for the model parameters.

Parameter	Value	Parameter	Value
AddPollen	Off	*MAX_BROODCELLS*	2000099
AllowReinfestation	Off	*MAX_HONEY_STORE_kg*	50
AlwaysDance	Off	*MAX_km_PER_DAY*	7299
ConstantHandlingTime	Off	*MergeWeakColonies*	Off
ContinousBroodRemoval	Off	*N_INITIAL_BEES*	10000
CRITICAL_COLONY_SIZE_WINTER	4000	*PollenIdeal*	Off
DroneBroodRemoval	Off	*ProbLazinessWinterbees*	0
EfficiencyPhoretic	0.115	*QueenAgeing*	Off
EfficiencyPhoretic2	0	*QueenSpaceTraveled*	Off
EggLaying_IH	On	*ReadBeeMappFile*	Off
Exp_feeding-schedule	Off	*ReadInfile*	Off
Exp_Honey-harvest	Off	*SeasonalFoodFlow*	On
Exp_input_initial_conditions	Off	*SHIFT_G*	-40
Exp_StartDay	0	*SHIFT_R*	30
Exp_X-Days	365	*Swarming*	No swarming
Experiment	none	*TIME_NECTAR_GATHERING*	1200
FeedBees	On	*TIME_POLLEN_GATHERING*	600
HoneyHarvesting	Off	*TreatmentDay*	270
HoneyIdeal	Off	*TreatmentDay2*	0
KillAllMitesInCells	Off	*TreatmentDuartion*	40
KillAllMitesInCells2	Off	*TreatmentDuration2*	0
KillOpenBrood	Off	*VarroaTreatment*	On
KillOpenBrood2	Off	*Weather*	Rothamsted (2009)

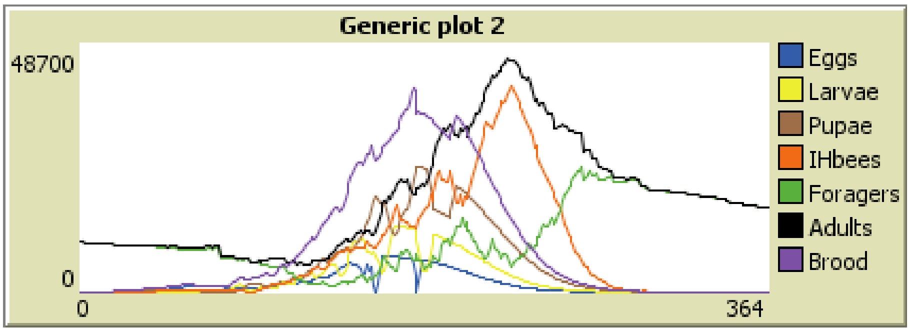
6.2.2. Module I - Rocket launch affected vs. default colony development

As described in Chapter 5.5.2., the module enabling inclusion in the simulation of the space travel effects considers only the rocket launch effects on honey bee queen and contains two response scenarios: low and high. Low response stands for the situation when the queen bee, in response to the stress associated with the rocket launch, limits itself in the number of laid eggs. The high response is contrary to the low one and pictures the scenario when the queen bee, which experienced space travel-related hypergravity, maximizes the number of eggs laid. Figure 13 presents the colony structure workers' graphs comparison for the default scenario, where the queen bee was not given any kind of stress associated with space travel, with low and high space travel response scenarios.

QueenSpaceTraveled: Off; *SpaceTravelType:* None; *SpaceResponse:* N/A



QueenSpaceTraveled: On; *SpaceTravelType:* Launch; *SpaceResponse:* High



QueenSpaceTraveled: On; *SpaceTravelType:* Launch; *SpaceResponse:* Low

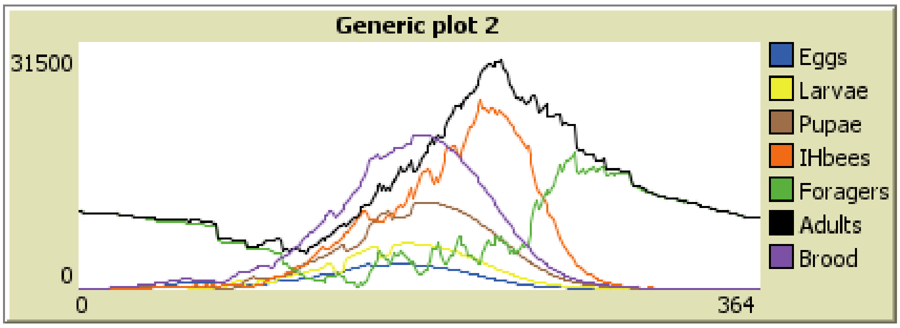
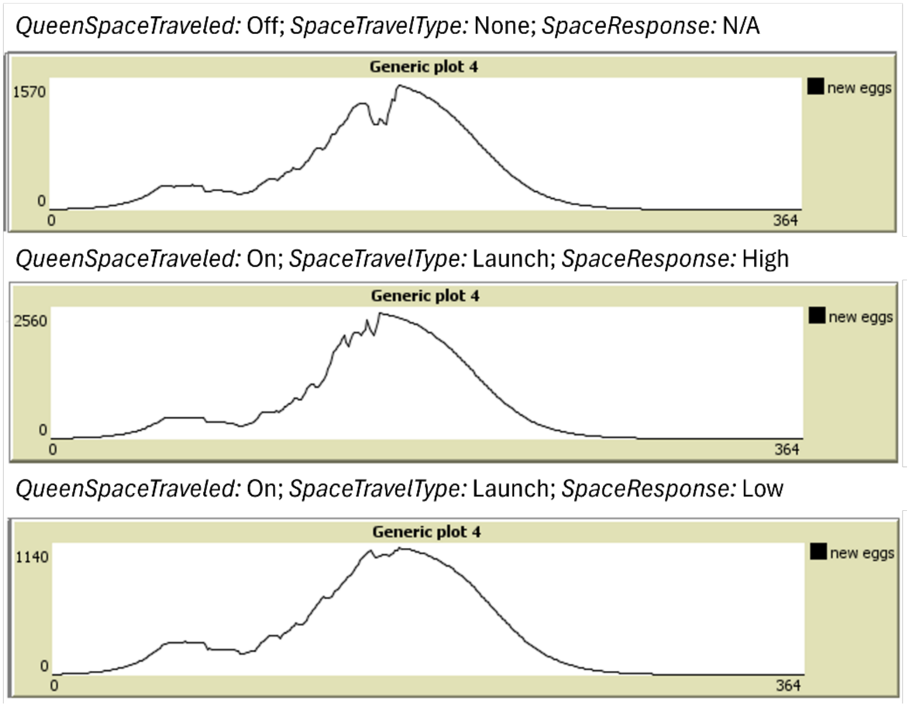


Fig. 13 Comparison of the colony structure for worker bee brood and adults for different flight response scenarios for default modeling conditions. (Source: BEEHAVE output plots, based on own data)


QueenSpaceTraveled: Off; *SpaceTravelType:* None; *SpaceResponse:* N/A

QueenSpaceTraveled: On; *SpaceTravelType:* Launch; *SpaceResponse:* High

QueenSpaceTraveled: On; *SpaceTravelType:* Launch; *SpaceResponse:* Low

Fig. 14 Comparison of the queen's egg laying rate for different flight response scenarios for default modeling conditions. (Source: BEEHAVE output plots, based on own data)

As can be seen in Figure 14, despite the space flight scenario chosen, the egg-laying rate of the queen follows an analogous pattern. What differs in various scenarios queens is the maximum number of eggs laid and minor fluctuations in the pattern preceding reaching the maximum.

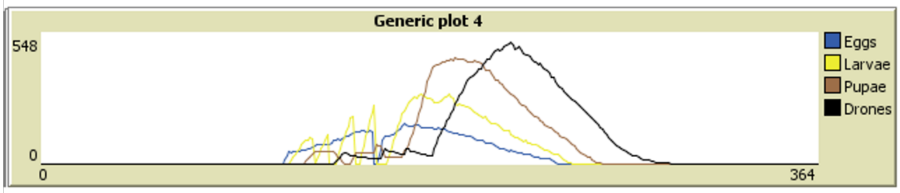
6.2.3. Module I – droning factor

As initially shown by the experiment described in Chapter 4 and [109], rocket launch-related acceleration impacts not only the overall egg-laying pattern of the queen but also the queen's droning pace. Stress' impact on reproduction is a known phenomenon in biology field for various insect species, such as *Heliconius erato* [110] or *Bicyclus anynana* [111]. Due to observing similar behavior during the described experiment, new global variables were defined (*SpaceTravelDroningFactor* describing how many more drone eggs are laid in a "space colony" and *SpaceTravelDroningShift* indicating the number of days by which the first drones' appearance in the colony will shift) and its basic values were established. Both low and high response scenarios for the droning factor were additionally parametrized.

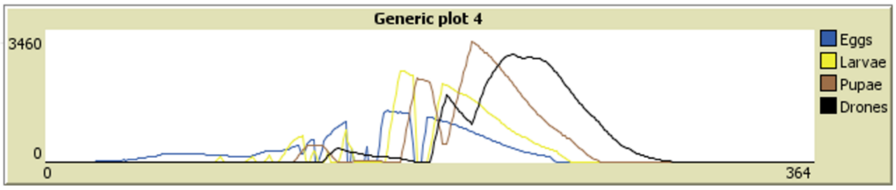
The high response scenario assumes the earlier appearance of the drones in the colony (indicated by the *SpaceTravelDroningShift*) as well as their greater total number (multiplied by *SpaceTravelDroningFactor* and parametrization factor equal 0.7). The low response scenario recalculates the basic SpaceTravelDroningFactor by a parametrization factor of 0.2 (which, as a result, gives a number < 1), according to the real-life data indicating such colony development pace. Additionally, it does not consider the shift in the appearance of first drones (*SpaceTravleDroningShift*), as no such event was observed in the experimental colonies.

The high response factors can be adjusted accordingly when more real-life response data become available. Parametrization factors will need to be adjusted accordingly for the high and low response scenarios to ensure simulation results are consistent with the actual observations.

QueenSpaceTraveled: Off; *SpaceTravelType:* None; *SpaceResponse:* N/A



QueenSpaceTraveled: On; *SpaceTravelType:* Launch; *SpaceResponse:* High



QueenSpaceTraveled: On; *SpaceTravelType:* Launch; *SpaceResponse:* Low

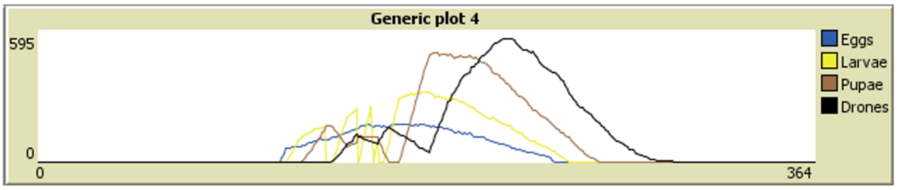


Fig. 15 Comparison of the number of drones in the colony, depending on the simulation scenario chosen. No colonies merging and honey harvesting procedures were considered, default start and weather conditions were applied. (Source: BEEHAVE output plots, based on own data)

Figure 15 presents the number of drones during the colony development for the default, high, and low space flight response scenarios – the maximum number of drones in the high response colony is almost seven times higher than for the non-space-traveled one.

6.2.4. Module I - Space travel and aging combined impact

Verification of the possibility of modeling the honey bee colony development for the aging queen, which was given to the space travel conditions, was performed. Model output graphs, which can be seen on Figure 16 and 17, compare four variants: (1) queen bee is not aging and was not given to the space travel conditions, (2) queen bee is aging but was not given to the space travel conditions, (3) queen bee is aging and was given to the space travel conditions with high space response, (4) queen bee is aging and was given to the space travel conditions with low space response.

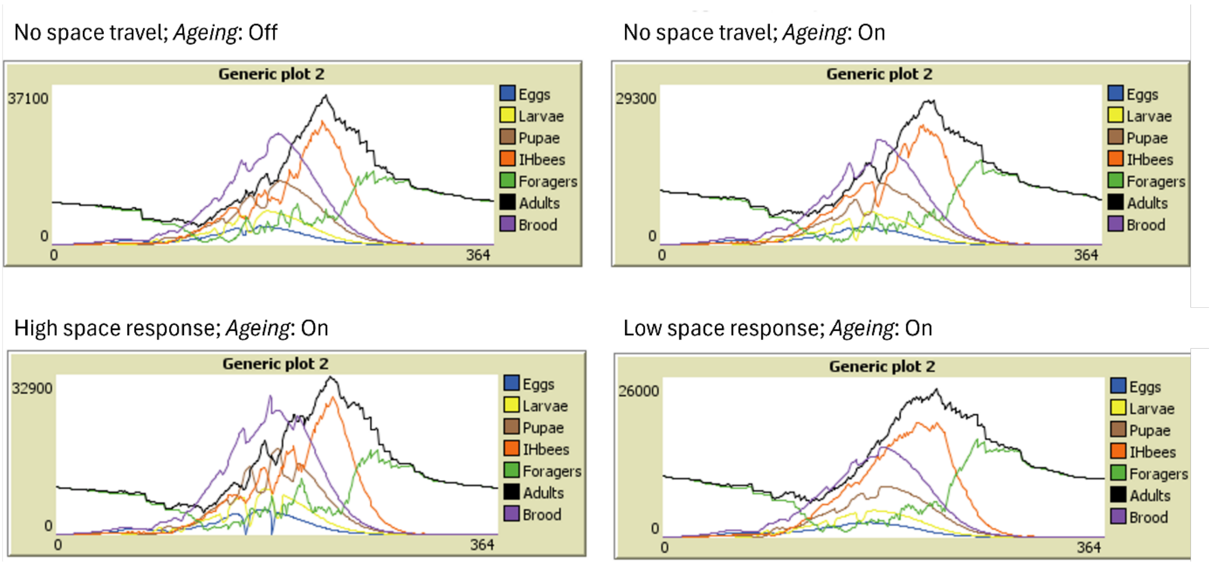


Fig. 16 BEEHAVE graphical output for various combinations of space responses and aging effect on colony development. (Source: BEEHAVE output plots, based on own data)

Aging, low space response colony is characterized by the lowest number of brood from all the considered variants. Worth noticing is the fact that for a high-response colony, the total number of worker adults is lower than for the default scenario with no space impact and aging considered (Fig.16), contrary to the egg-laying rate presented in Fig 17. Such a counter-intuitive observation might indicate worker egg development issues and/or increased development mortality related to the queen's aging.

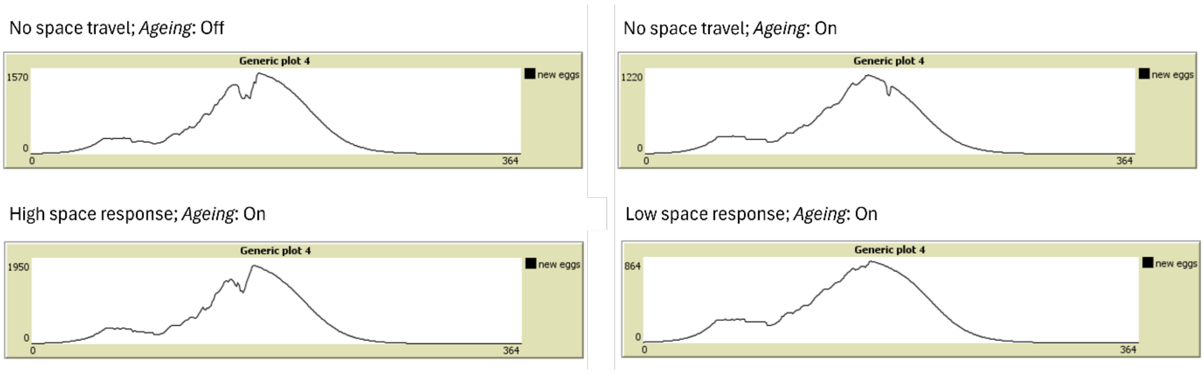


Fig. 17 . BEEHAVE graphical output for various combinations of space responses and aging effect on queen fertility represented as the queen's egg-laying rate. (Source: BEEHAVE output plots, based on own data)

As can be observed in Fig. 17, a combination of low response space conditions impact and aging on the predicted egg-laying pattern of the queen generates the greatest difference in the appearance in comparison with the simulation where no aging nor space conditions are considered. However, queens in all the considered scenarios follow the same pattern in terms of the trend in the number of eggs laid.

6.2.5. <u>Module I – simulation starting day vs. space travel colony development</u>

The outputs of the model were compared for two variables: the chosen simulation starting day and the effects of space conditions for both scenarios. As can be seen in Fig. 18, results vary slightly for each combination of variables, tending to predict the greatest number of adult worker bees for simulation starting on the 60th day, with the tendency to drop again starting on the 120th day. Such results are in line with the current beekeeping expertise, recognizing the emergence from overwintering as one of the most fragile moments for the later colony development [112]. At the same time, the postponed start of the development prevents the colony from reaching its full potential due to limited resources and increased competition [113].

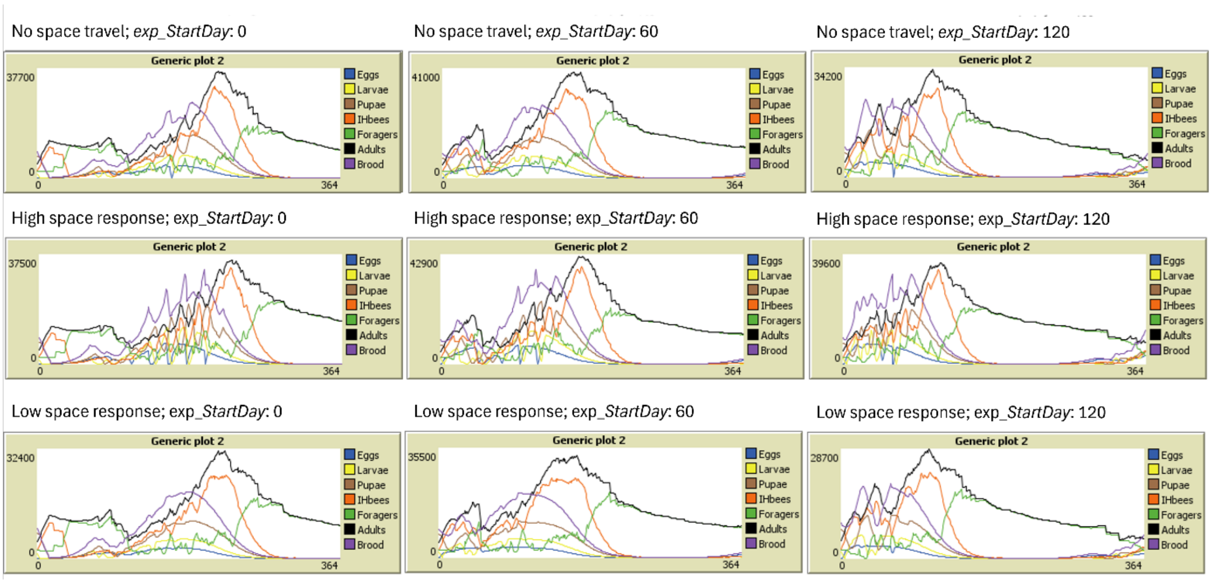
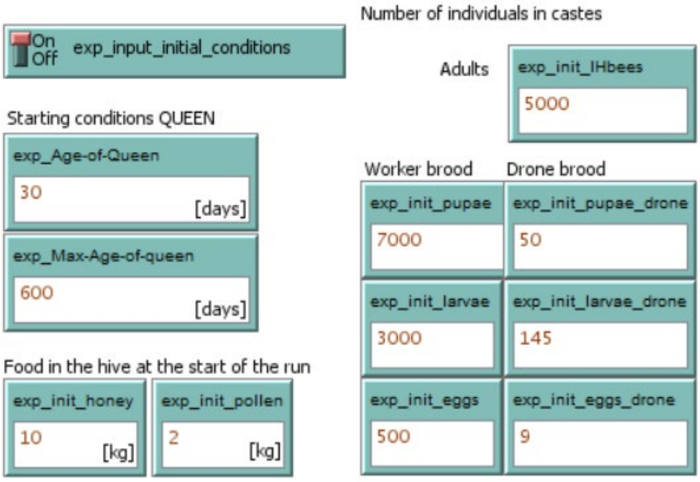

No space travel; *exp_StartDay*: 0
No space travel; *exp_StartDay*: 60
No space travel; *exp_StartDay*: 120
High space response; exp_*StartDay*: 0
High space response; exp_*StartDay*: 60
High space response; exp_*StartDay*: 120
Low space response; exp_*StartDay*: 0
Low space response; exp_*StartDay*: 60
Low space response; exp_*StartDay*: 120

Fig. 18 Colony development dynamics comparison in terms of the simulation starting day. The same starting conditions were considered for all the scenarios. (Source: BEEHAVE output plots, based on own data)


Number of individuals in castes

Adults

exp_init_IHbees: 5000

Worker brood

exp_init_pupae: 7000

exp_init_larvae: 3000

exp_init_eggs: 500

Drone brood

exp_init_pupae_drone: 50

exp_init_larvae_drone: 145

exp_init_eggs_drone: 9

Starting conditions QUEEN

exp_Age-of-Queen: 30 [days]

exp_Max-Age-of-queen: 600 [days]

Food in the hive at the start of the run

exp_init_honey: 10 [kg]

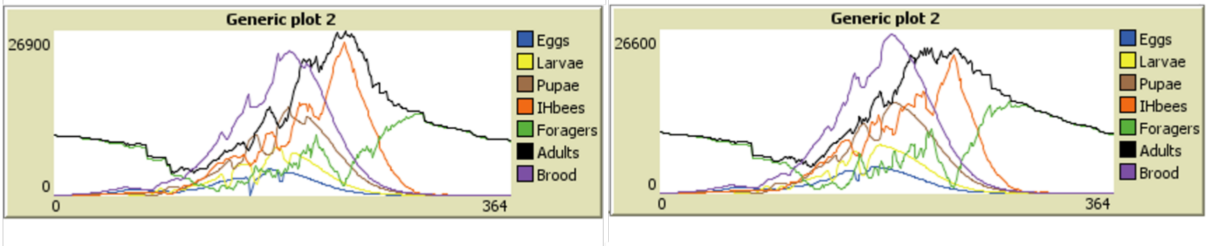
exp_init_pollen: 2 [kg]

Fig. 19 Altered simulation starting day initial conditions. Identical values were used for all the scenarios considered. (Source: BEEHAVE model)

6.2.6. Module III – hive type independent analysis

Hive-type changes have a direct impact on the maximum number of cells available for both egg-laying and storing food. The introduced module was analyzed in terms of the overall impact on the honey bee colony development. Additionally, the functions strictly related to the change are merging too weak colonies, preventing them from death but causing sudden increases in the total number of adult worker bees and honey harvesting, freeing space for more honey to be harvested, therefore for more workers to be foraging, but simultaneously to introduce additional variable in the analysis.

Four various scenarios were compared: (1) colonies merging and honey harvesting were on with default maximum honey stores value of 50 kg and default maximum number of cells available for brood (2,000,099 cells), (2) colonies merging and honey harvesting were on, maximum honey stores value (27.6 kg) and cells available for the 4-boxes mini plus hive type was used, (3) colonies merging, and honey harvesting were off with default maximum honey stores value and cells available, (4) colonies merging, and honey harvesting were off, maximum honey stores value for the 4-boxes mini plus hive type and a number of cells for brood were used. For all the options, the beekeeping scenario with the feeding option was on as well. No space travel impact was considered in those scenarios.



MergeWeakColonies: On; *HoneyHarvesting*: On; *FeedBees*: On
MAX_HONEY_STORE_kg: 27.6; *MAX_BROODCELLS*: 55200
31.12.: HONEY STORE [kg]: 16.9

MergeWeakColonies: On; *HoneyHarvesting*: On; *FeedBees*: On
MAX_HONEY_STORE_kg: 50; *MAX_BROODCELLS*: 2000099
31.12.: HONEY STORE [kg]: 20.3

MergeWeakColonies: Off; *HoneyHarvesting*: Off; *FeedBees*: On
MAX_HONEY_STORE_kg: 50; *MAX_BROODCELLS*: 2000099
31.12.: HONEY STORE [kg]: 37.9

MergeWeakColonies: Off; *HoneyHarvesting*: Off; *FeedBees*: On
MAX_HONEY_STORE_kg: 27.6; *MAX_BROODCELLS*: 55200
31.12.: HONEY STORE [kg]: 16.8

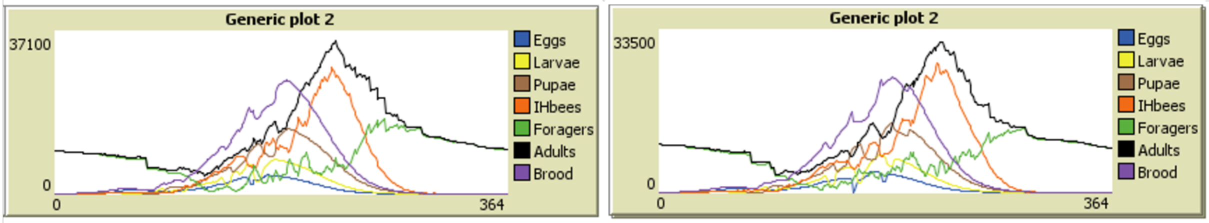


Fig. 20 BEEHAVE graphical output for various beekeeping procedures for two different hive type scenarios. (Source: BEEHAVE output plots, based on own data)

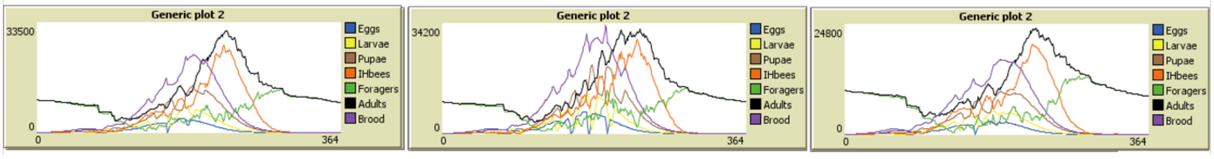
Figure 20 shows that for the default hive with no honey harvesting scenario, the total number of adults is the greatest. Worth noticing is the fact that the total number of adult workers is lower for both scenarios, considering weak colonies merging. Most probably it is caused by including honey harvesting in the scenarios, causing the amount of food available for workers to be on lower level than for the non-merging scenarios and additionally proves that the strength of the colony is strongly related to the amount of available food.

The size of the hive has a lower impact on the overall size of the colony in case of weak colonies merging and honey harvesting due to external limitations of the available resources. In case of not harvesting honey, space available in the beehive has a more visible impact on the colony size.

6.2.7. Space conditions and hive type

The cooperation of the space conditions module and hive-type module was verified. Due to the methodology of the biological experiment performed, as described in the Chapter 4, as well as due to more visible changes being noted in the case of the scenario not considering weak colonies merging and honey harvesting, as shown in the previous chapter, space travel scenarios were performed exclusively for no merging, no harvesting scenario.

DEFAULT beekeeping ; *HiveType*: mini-plus; *exp_StartDay*: 0; *MergeWeakColonies:* Off; *HoneyHarvesting*: Off; *MAX_HONEY_STORE_kg*: 27.6; *MAX_BROODCELLS*: 55200



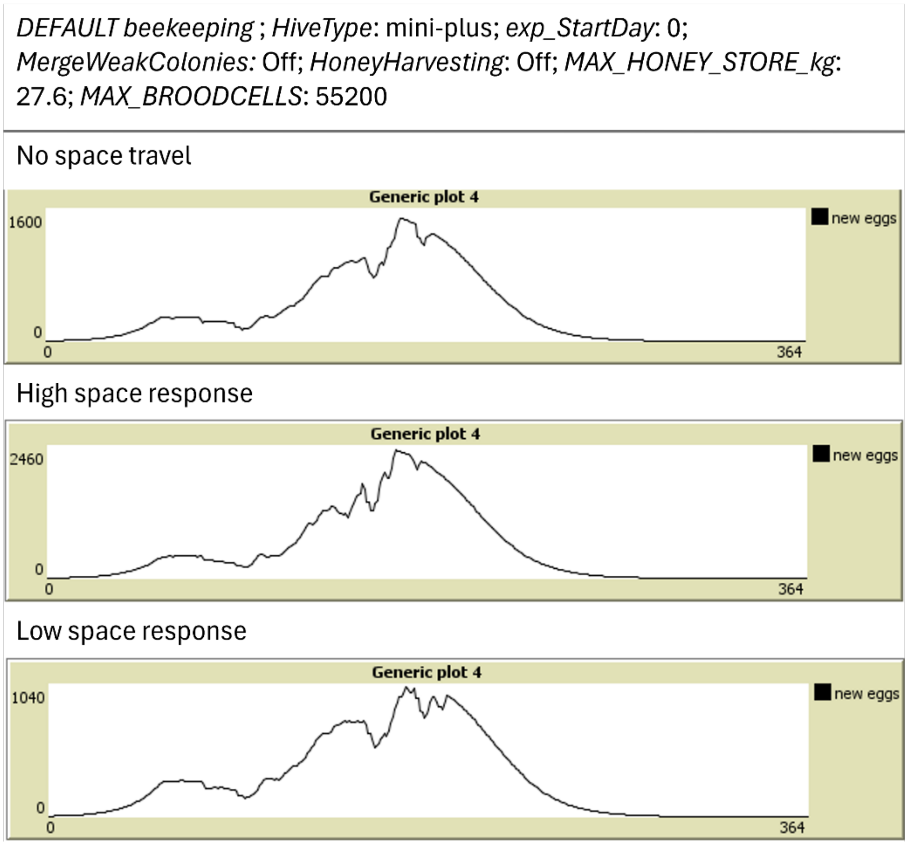
No space travel

High space response

Low space response

Fig. 21 Model's colony development predictions for combination of space travel impact with beehive type change. (Source: BEEHAVE output plots, based on own data)

As can be seen in Fig. 21, the model's responses in the case of combined simulation are within expected ranges and vary for different space and non-space responses. Predictions for the high-response space travel scenario present the least stability among analyzed scenarios. Moreover, more artifacts are being noticed, unrelated to the actual queen bee behavior. Last but not least, the maximum number of adult workers within the colony is predicted to be less than for the non-space scenario (32,600 vs. 33,500), which is contrary to the expectations, as a high response scenario shall predict honey bee queen to lay more eggs than in non-space travel situation. Such a small difference between default and high response scenarios with consideration of the predicted egg-laying rate, presented in Figure 21, can be explained by the bigger number of the brood requiring increased energy expenditure on hive thermoregulation. Considering that cooling hive requires more energy than warming it, more food intake is required by worker bees. Cells which are emptied in the process are in turn immediately used by the queen for laying new eggs which limits the space for new food to be stored. All these events, combined with the limited volume of the hive, lead to the results predicted by the model – lowering number of adult worker bees in the colony despite the greater productivity of the queen in terms of the number of laid eggs.



DEFAULT beekeeping ; *HiveType*: mini-plus; *exp_StartDay*: 0; *MergeWeakColonies:* Off; *HoneyHarvesting*: Off; *MAX_HONEY_STORE_kg*: 27.6; *MAX_BROODCELLS*: 55200

No space travel

High space response

Low space response

Fig. 22 Egg-laying rate model's outputs comparison for three space travel scenarios for mini-plus hive type. (Source: BEEHAVE output plots, based on own data)

On the last day of the simulation, the 31st of December, the high response colony was the greatest, with a size of 9,875 specimens, while at the same time, its honey stores were the smallest, at 15.8 kg. The low response colony was the smallest, with the greatest amount of food stores of 17.7 kg. Such a disproportion comes from the limited size of the hive, forcing the colony to make a tradeoff between their number versus the space left for the food stores.

6.3. Real-life data

6.3.1. Weather data source and preparation

Data on the weather during the data gathering period comes from the Institute of Meteorology and Water Management, National Research Institute, and includes the period from August 1, 2021, to May 31, 2022, covering the entire time of the field study. Weather data contains information on the maximum daily temperature and number of hours with sunlight. The meteorological station from which temperature data was obtained was the Kraków-Balice station (identification number: 566; GPS: 50°05', 19°48'), localized 7.15 km from the experimental apiary, SWW direction. Data on the number of hours with sunlight comes from the station Kraków-Wola Justowska, localized 0.7 km from the apiary, SSW direction. Any gaps in data from the aforementioned stations were filled with information from Kraków-Obserwatorium station, localized 4.15 km away from the experimental apiary in the East direction. Fig. 23 presents the localization of the apiary and chosen meteorological stations.

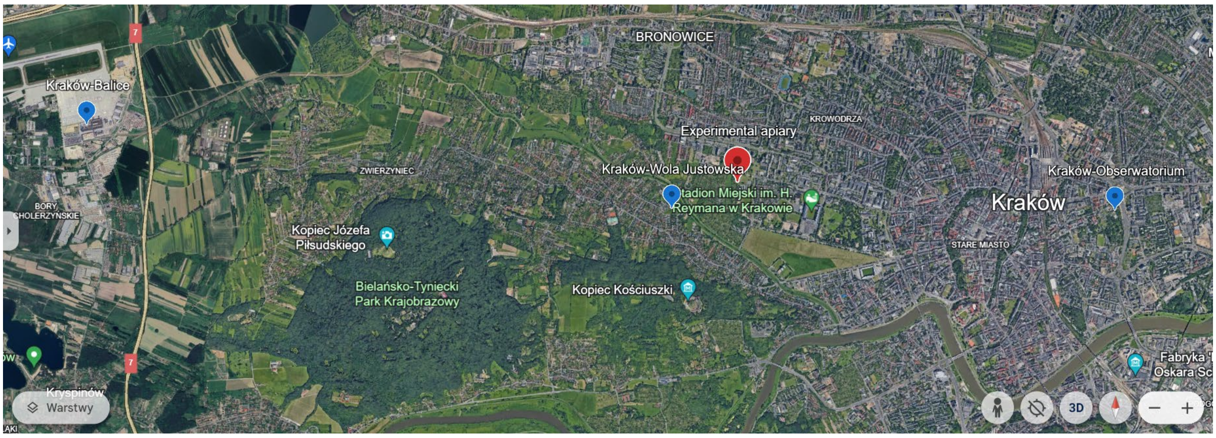


Fig. 23 Map of meteorological stations from which the weather data was acquired (marked with blue pins) with the experimental apiary localization marked (red pin). (source: Google Earth)

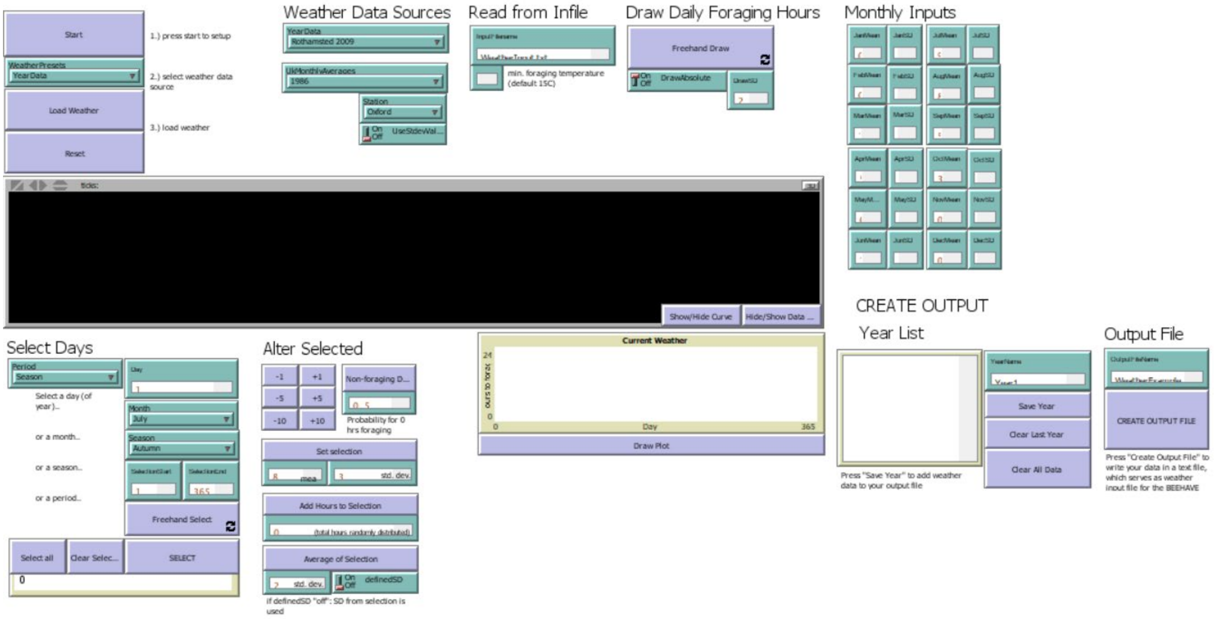


Fig. 24 Interface of the BEEHAVE_Weather (Source: BEEHAVE_Weather)

Obtained data were prepared for uploading to the BEEHAVE_Weather tool, of which the interface can be seen in Fig. 24, enabling recalculation of the weather data to information suitable for further analysis of the BEEHAVE model. For that, acquired information was merged into a single text file containing three columns, respectively: the day of the year, the maximum temperature measured, and a total number of sun hours. Due to the BEEHAVE_Weather limitations, such file had to be prepared separately for the data from 2021 and 2022.

6.3.2. Real-life food stores data preparation

Analogous to Chapter 5.5.3, data on the amount of food stores was recalculated to be expressed in mass rather than the number of cells occupied by it. Such a transition enabled comparison of the model outputs with gathered experimental data, presented in the following chapters.

The recalculation assumed that pollen's weight is 8% of the total food source weight in the hive [107], [108]. Adopting values proposed by Schmick and Crailsheim [71] and Camazine et.al [114] equivalent to the cell mass occupied entirely by pollen (0.23 mg) and by nectar (0.5 mg) enabled to estimate that 19% of cells marked as occupied by food stores were filled with pollen, and 81% - by nectar. Such values were adopted in previous real-life model validation studies [75].

6.3.3. Local weather data impact on simulation

Data from the biological research described in Chapter 4 covers a very specific period of the year. Due to that reason, verification of simulation differences between the real-life weather data and the default weather file used by the BEEHAVE was performed. The simulation starting day was set to 213, and it was run for an equivalent of 303 days.

Simulation starting conditions were set. The mean value of the real-life data from the control sample from the mentioned research was used. A number of adult workers was established on the basis of the brood data and considering brood to worker ratio investigated by Eckert et al. [115]. For the given case, it was calculated that per one brood cell, 1.7 adult worker bees are present in the colony.

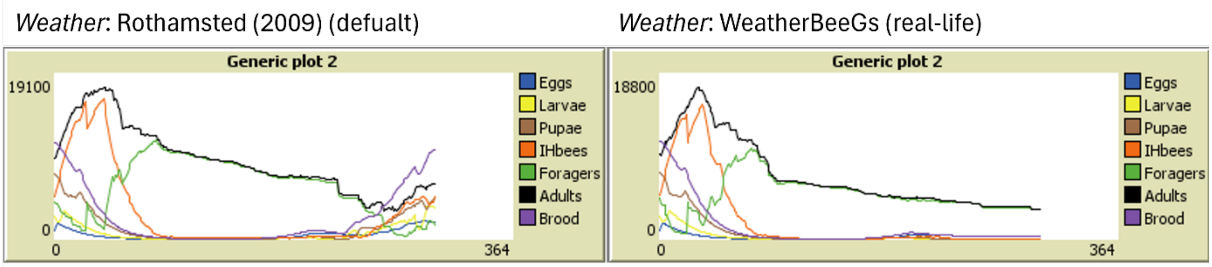
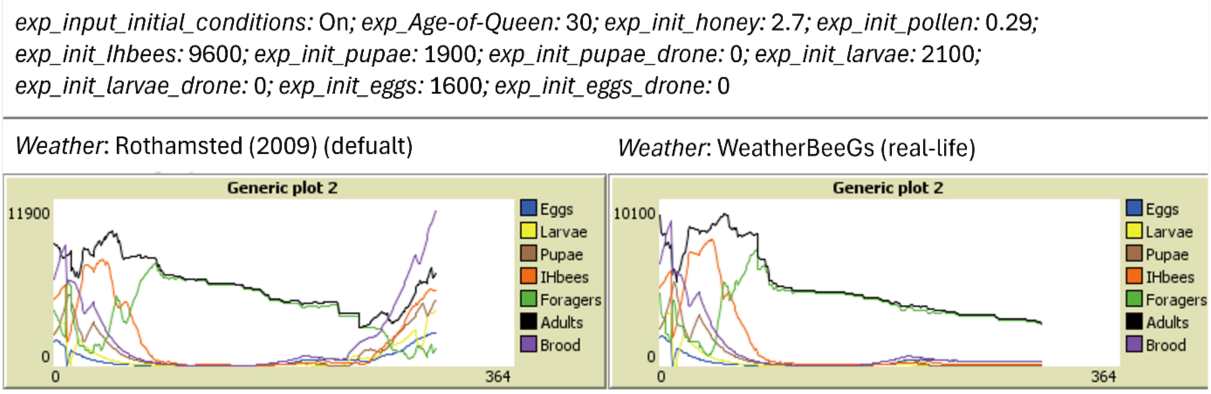

Weather: Rothamsted (2009) (defualt)
Weather: WeatherBeeGs (real-life)

Fig. 25 Comparison of the simulation graphical outputs for default simulation initial conditions of colony structure. (Source: BEEHAVE output plots, based on own data)


exp_input_initial_conditions: On; *exp_Age-of-Queen:* 30; *exp_init_honey:* 2.7; *exp_init_pollen:* 0.29; *exp_init_Ihbees:* 9600; *exp_init_pupae:* 1900; *exp_init_pupae_drone:* 0; *exp_init_larvae:* 2100; *exp_init_larvae_drone:* 0; *exp_init_eggs:* 1600; *exp_init_eggs_drone:* 0

Weather: Rothamsted (2009) (defualt)
Weather: WeatherBeeGs (real-life)

Fig. 26 Comparison of the simulation graphical outputs for simulation initial conditions of the colony structure numbers characteristic for the experimental control sample. (Source: BEEHAVE output plots, based on own data)

As can be seen in Fig. 26, for the real-life colony structure, the model based on the real-life weather data predicts slightly better colony performance in terms of the maximum number of adult workers at the end of the first year. On the contrary, as can be seen in Fig. 25, for the default colony structure input data, a number of adult worker bees is greater than for default weather data. Such an observation is in line with the common knowledge on the development adaptation of a honey bee colony to external conditions.

Nevertheless, colony development starts after the overwintering for both initial conditions cases is predicted to be significantly delayed for the real-life weather data in comparison with the simulation default weather conditions. The observation enables us to conclude that weather conditions in Kraków were unfavorable at the beginning of the second year in terms of colony development, causing a delay in the queen bee's resumption of egg-laying.

6.4. Model predictions vs. experimental data comparison

6.4.1. Simulation default conditions

For simulations presented in the following subchapters, starting conditions presented in Table 5 were applied.

*Table 5 Real-life data simulation initial conditions. Values changed in all simulations in comparison to table 4 were **bolded**. Values changed for specific simulation's sake were underlined.*

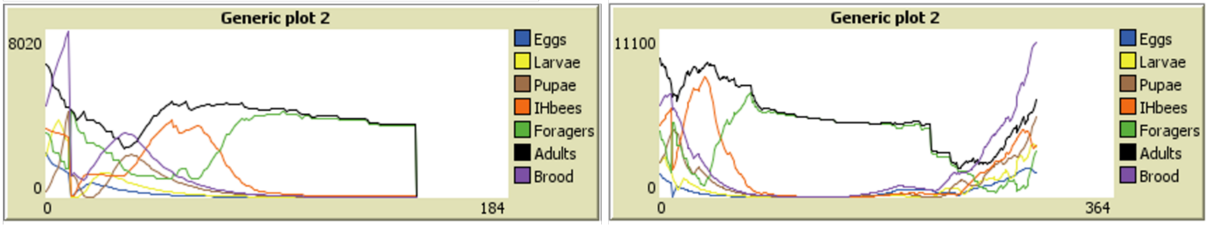
Parameter	Value	Parameter	Value
AddPollen	Off	***MAX_BROODCELLS***	**55200**
AllowReinfestation	Off	***MAX_HONEY_STORE_kg***	**27.6**
AlwaysDance	Off	*MAX_km_PER_DAY*	7299
ConstantHandlingTime	Off	*MergeWeakColonies*	Off
ContinousBroodRemoval	Off	*N_INITIAL_BEES*	10000
CRITICAL_COLONY_SIZE_WINTER	4000	*PollenIdeal*	Off
DroneBroodRemoval	Off	*ProbLazinessWinterbees*	0
EfficiencyPhoretic	0.115	*QueenAgeing*	Off
EfficiencyPhoretic2	0	*QueenSpaceTraveled*	Off
EggLaying_IH	On	*ReadBeeMappFile*	Off
Exp_feeding-schedule	Off	*ReadInfile*	Off
Exp_Honey-harvest	Off	*SeasonalFoodFlow*	On
Exp_input_initial_conditions	**On**	*SHIFT_G*	-40
Exp_StartDay	**221**	*SHIFT_R*	30
Exp_X-Days	**264**	*Swarming*	No swarming
Experiment	none	*TIME_NECTAR_GATHERING*	1200
FeedBees	On	*TIME_POLLEN_GATHERING*	600
HoneyHarvesting	Off	*TreatmentDay*	270
HoneyIdeal	Off	*TreatmentDay2*	0
KillAllMitesInCells	Off	*TreatmentDuartion*	40
KillAllMitesInCells2	Off	*TreatmentDuration2*	0
KillOpenBrood	Off	*VarroaTreatment*	On
KillOpenBrood2	Off	*Weather*	Rothamsted (2009)

6.4.2. Control sample hive composition field data vs. model's predictions

6.4.2.1. Default weather data

Model outputs were graphically compared to the real-life data described in the Chapter 4. No statistical analysis is presented as gathered data on hypergravity's impact on honey bee queen egg-laying capabilities is very limited, and therefore, it is appropriate only to describe observed trends.

First, the verification of the model's ability to predict the winter survival of the colony was performed. Results can be seen in Fig. 27, which shows the complications in the correct work of the model in case the simulation starts covering the real data gathering start. That might be caused by the time necessary for the bee colony to restart normal functioning after relocation, which directly preceded the beginning of the data collection.


exp_StartDay: 216 (4th of August)

exp_StartDay: 221 (9th of August)

Fig. 27 Comparison of model's outputs for various simulation starting day. (Source: BEEHAVE output plots, based on own data)

Because of the model's output for the August 4 scenario discussed above, it was decided to start the simulation on the next date with real-life data available, being August 9. Starting day (9th of August, 221st day of the year), the colony composition for each examined colony is presented in Table 6.

Table 6 Colony-specific starting conditions for simulations starting on day 221

		High space response (3K)	**Low space response (3V)**	**Control group (1D)**	**Control group (1H)**	**Mean real-life control data**
Workers	**Eggs**	2799	1786	979	2246	1613
	Larvae	2995	2246	1786	2477	2131
	Pupae	1267	1498	2131	1786	1958
	Adult bees	12201	9400	8323	11065	9694
Drones	**Eggs**	0	0	0	0	0
	Larvae	0	0	0	0	0
	Pupae	115	0	0	0	0
Honey [kg]		2.1508	1.5163	3.1026	2.4494	2.7760
Pollen [kg]		0.2321	0.1636	0.3348	0.2643	0.2995

Data range from that day up to the end of available data on 29th of April 2022 was compared with the simulation output. The simulation started on the day no. 221 and lasted for 264 days. The observed simulation behavior might be an indication that colony survival depends strongly on the number of adult bees and availability of the pupae (on August 4, no pupae were present in the colony).

A comparison of the real-life data available for two colonies representing the control sample, the mean value of the control sample response and the model's colony development prediction can be seen in Fig. 28 (below).

Due to severe limitations of available experimental data, the period compared on the provided graphs covers 50 days, starting the comparison on August 9th and ending on September 27th, being the last day of observations before colonies overwintering.

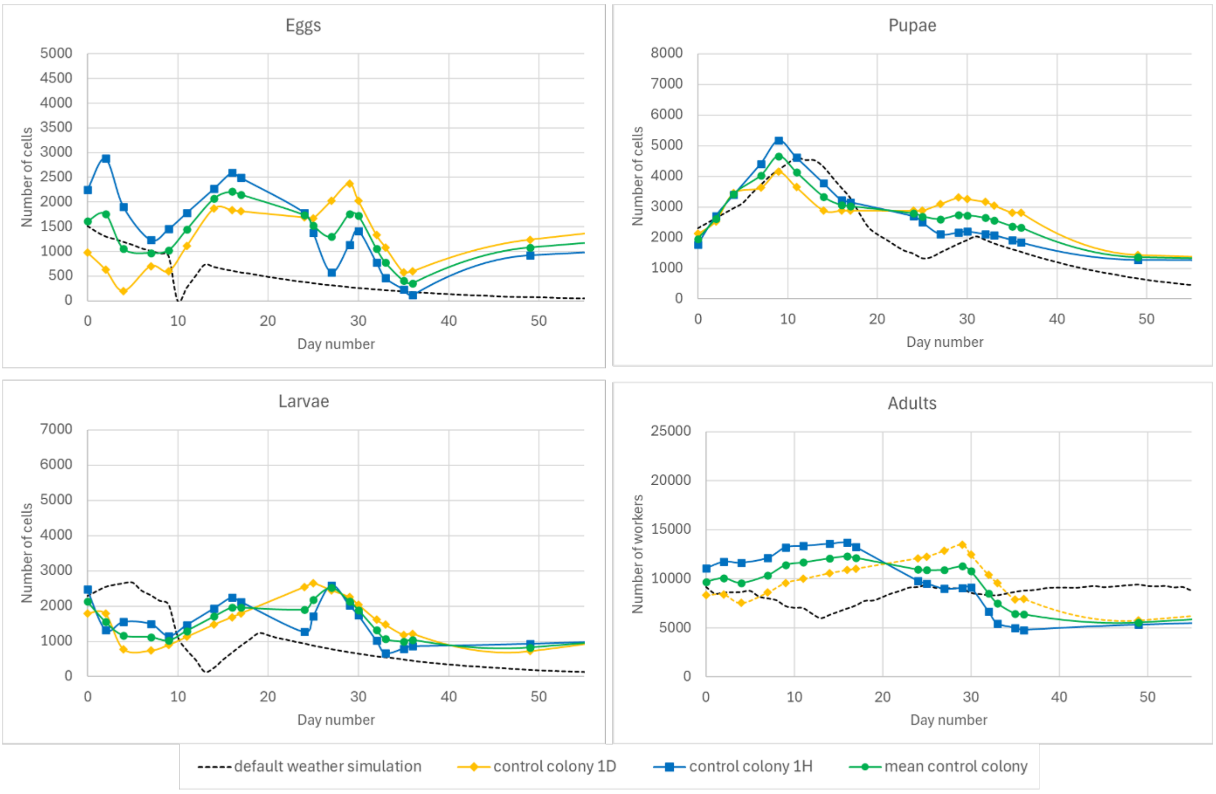


Fig. 28 Comparison of real-life measurements data points with the prediction of the BEEHAVE model. (Source: own materials)

The noticeable is the fact that the most accurate prediction has been made for the pupae–development dynamics and scale, which is visually very similar to the real-life data. The least accurate prediction concerns eggs. Model data have an artefact on the 11th day of the simulation. While simulation predicts a constant drop in the number of eggs towards the 55th day of the simulation, real-life measurements showed the last increase in that number before the overwintering. Such a difference might come from not including the real-life weather data on that stage of the simulation.

The comparison based on the greatest uncertainty data is presented in the last graph (Adults), which shows the number of adult workers available in the colony. This particular comparison's data is based on several assumptions, such as the one presented in subchapter 6.3.2., as no data on the number of adult workers was gathered during the study.

6.4.2.2. Real-life weather data

Considering a variation in the model caused by the inclusion of the real-life weather data, visible in Fig. 29, an analogous analysis was performed for the control sample initial composition data with the inclusion of the real-life weather data in the model.

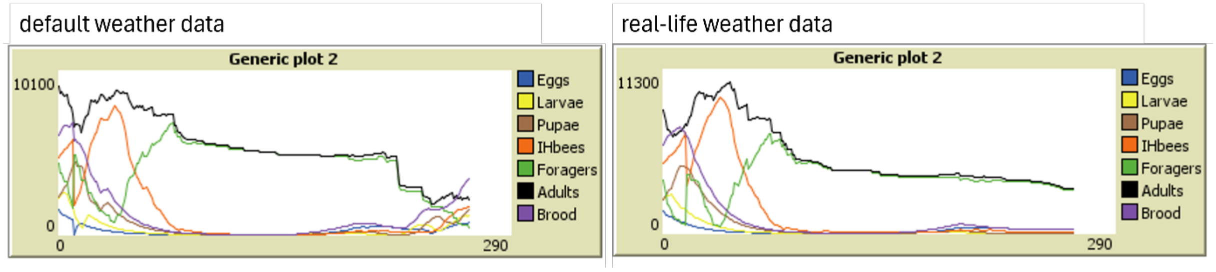


Fig. 29   Model's output comparison for model considering and not considering real-life weather data. (Source: BEEHAVE output plots, based on own data)

As presented in Fig. 30, predictions were the closest to the real-life data for pupae and the least for eggs. However, compared to the previous case, the model is less accurate in terms of trends, and predictions are more general without periodical development dynamics changes (Fig. 30 larvae and pupae graphs are smoother while considering real-life weather data in comparison with Fig. 28).

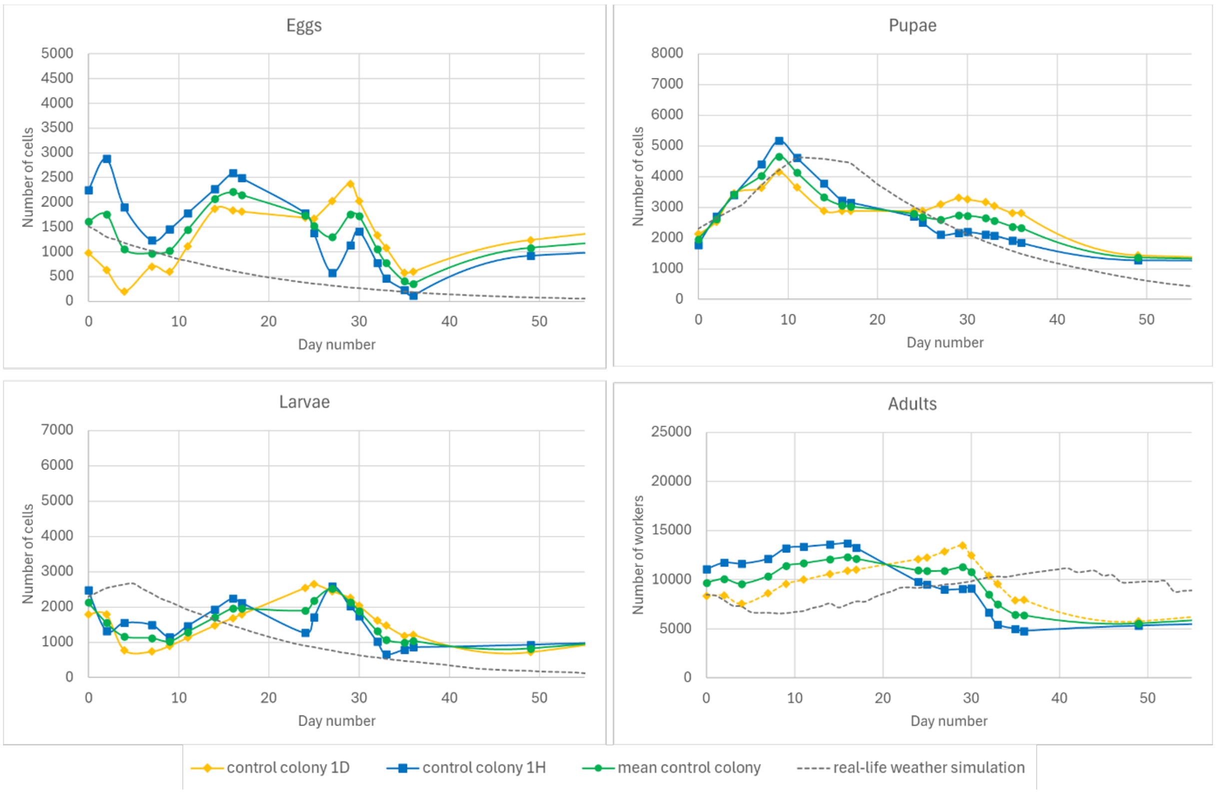


Fig. 30   Comparison of real-life measurements data points with the prediction of the BEEHAVE model considering real-life weather data. (Source: own materials)

6.4.3. Rocket launch altered colony development vs. default model's predictions

6.4.3.1. Low response scenario

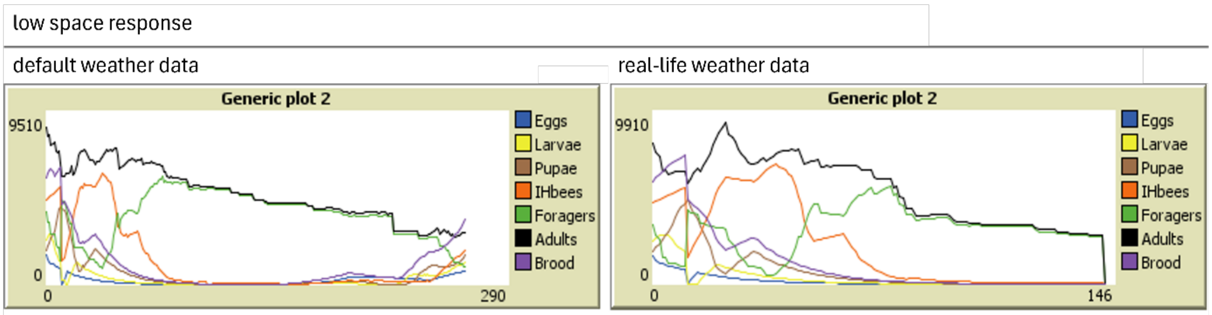


Fig. 31 Low space response scenario model output comparison for real-life and default weather data. (Source: BEEHAVE output plots, based on own data)

As shown in Fig. 31, development dynamics predicted by the model considering and not considering real-life weather data varies slightly. The most important difference is colony collapse prediction by the model using real-life weather data for the simulation. For that reason, real-life colony development data was compared with both outputs for the period before the overwintering.

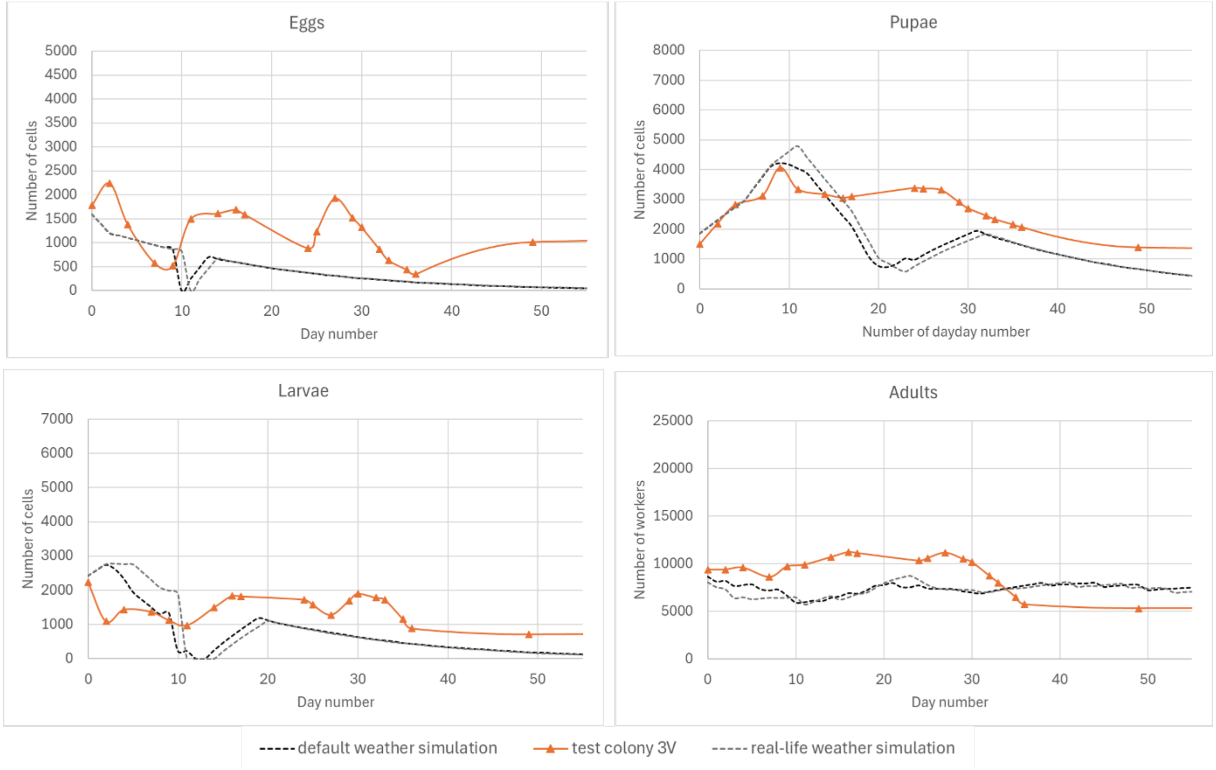


Fig. 32 Model output comparison for real-life and default weather data with respect to the specific bee development stage. (Source: own materials)

Similarly to the previous case, simulation output is the most similar to real-life data on the number of pupae, and predictions are least similar in the case of eggs.

6.4.3.2. High response scenario

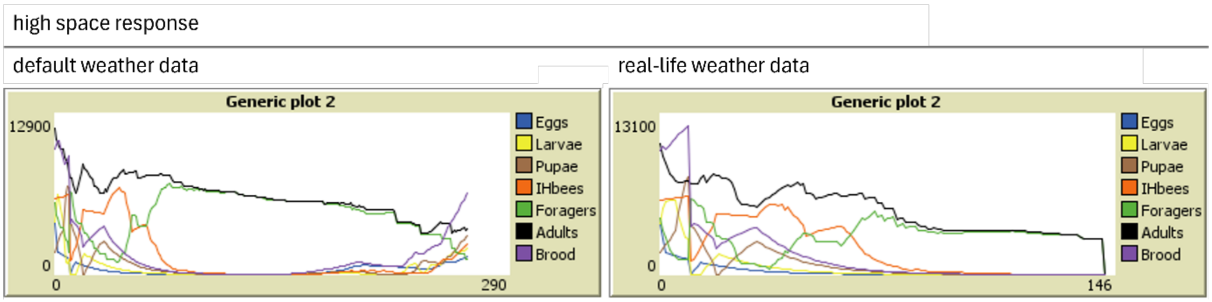


Fig. 33 High space response scenario model output comparison for real-life and default weather data. (Source: BEEHAVE output plots, based on own data)

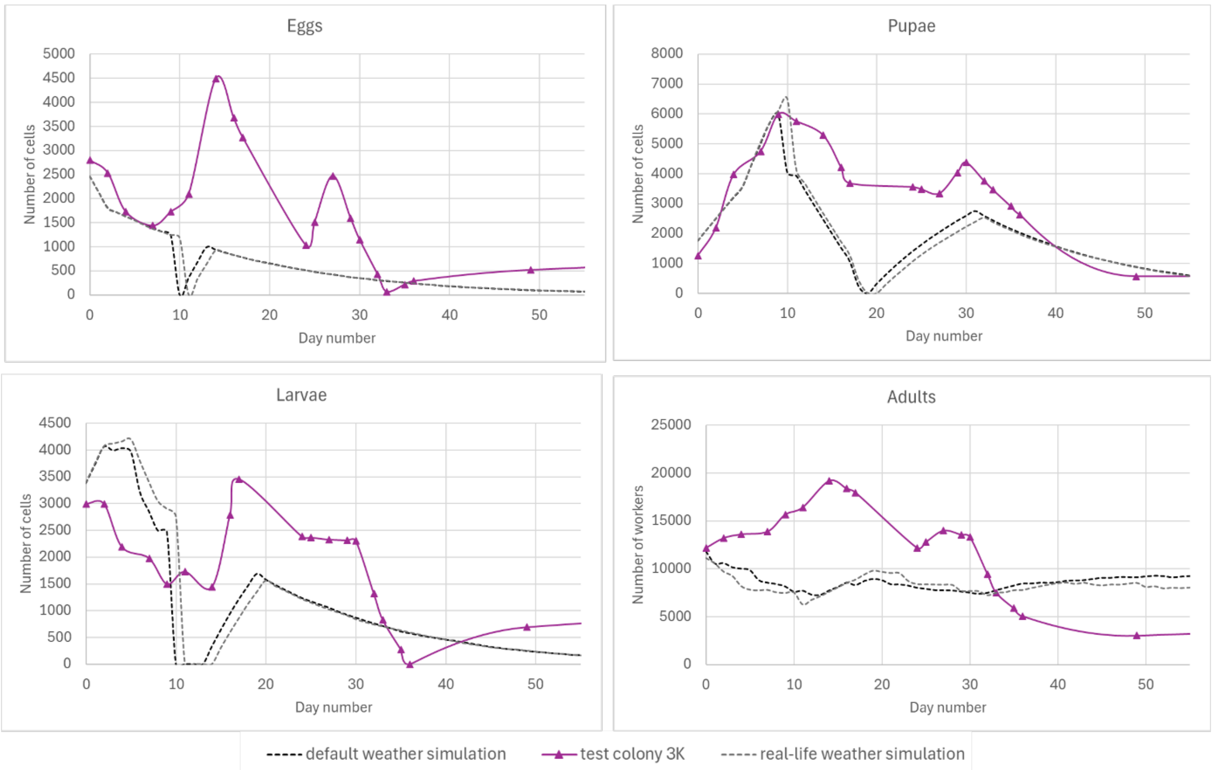


Fig. 34 High space response model output comparison for real-life and default weather data with respect to the specific bee development stage. (Source: own materials)

Similarly to the low-response scenario, development dynamics predicted by the model considering and not considering real-life weather data varies to a very limited extent in the case of a high space response model scenario. The most important difference, like in the case of the low-response scenario, is colony collapse prediction by the model using real-life weather data for the simulation, and no such event predicted for the model based on default weather data (Fig. 33). The more advanced bee development stage, the greater the difference in model's predictions – while for the number of eggs, predicted value for default and real-life weather conditions are virtually the same, for number of adult bees the difference is clearly visible. For that reason, real-life colony development data was compared with both outputs (Fig. 34). In this case the more visible is also an inverse prediction of the number of adults in the colony.

6.4.4. Real-life weather data and feeding schedule – all real-life input data considered

All the real-life data gathered during the experiment described in Chapter 4 was collected and used as input data for the model. The data considered were:

- Hive type
- Feeding schedule and added fondant mass
- Treatment timeline (with default efficiency phoretic)
- Queen age at the beginning of the simulation
- The number of individuals in the castes

Control, high, and low space response colonies data was compared with the simulation outcomes in the next chapters, considering both the real-life weather and default weather scenario.

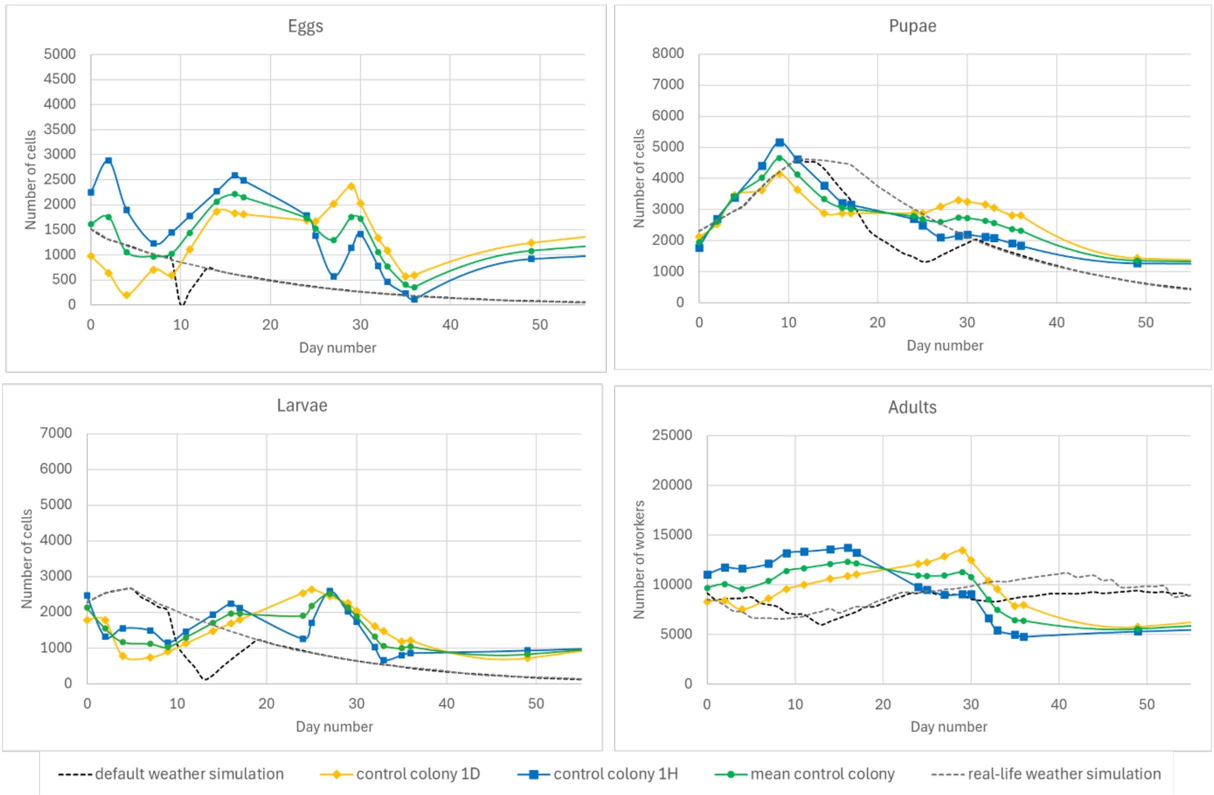


Fig. 35 Control sample real-life data and simulation outcomes comparison. (Source: own materials)

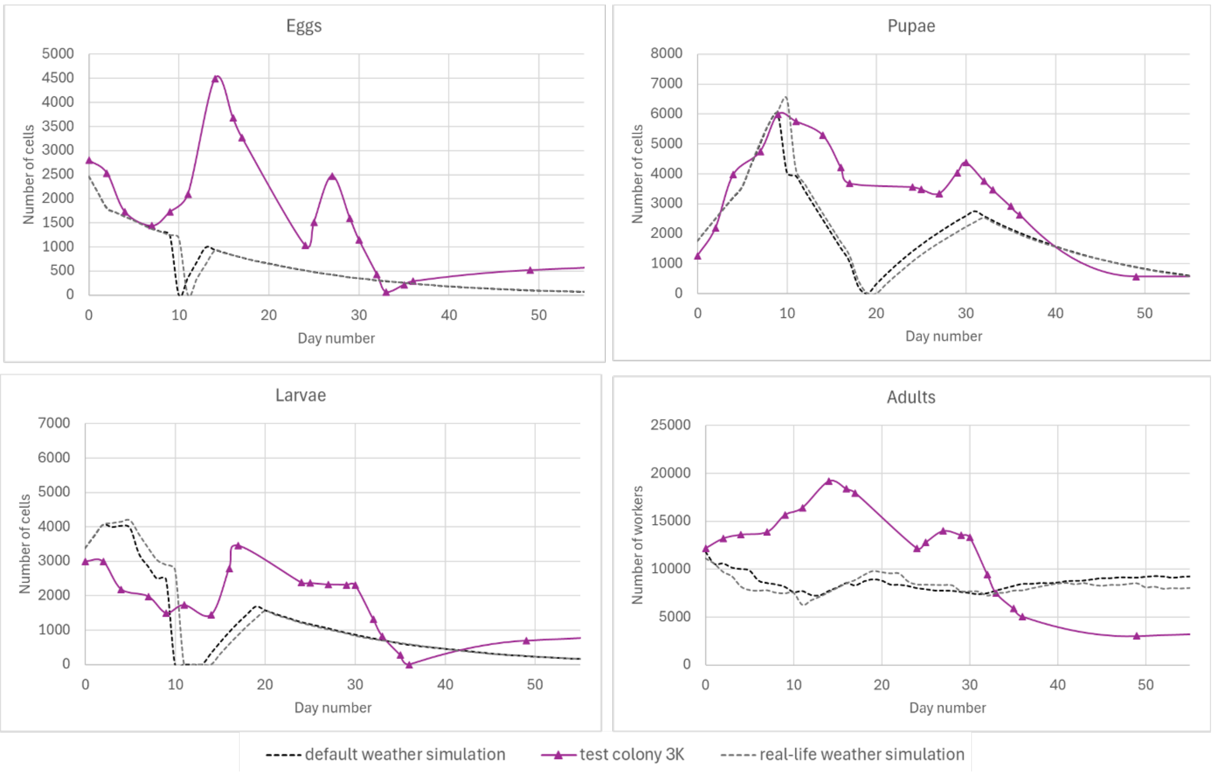


Fig. 36 High space response sample real-life data and simulation outcomes comparison. (Source: own materials)

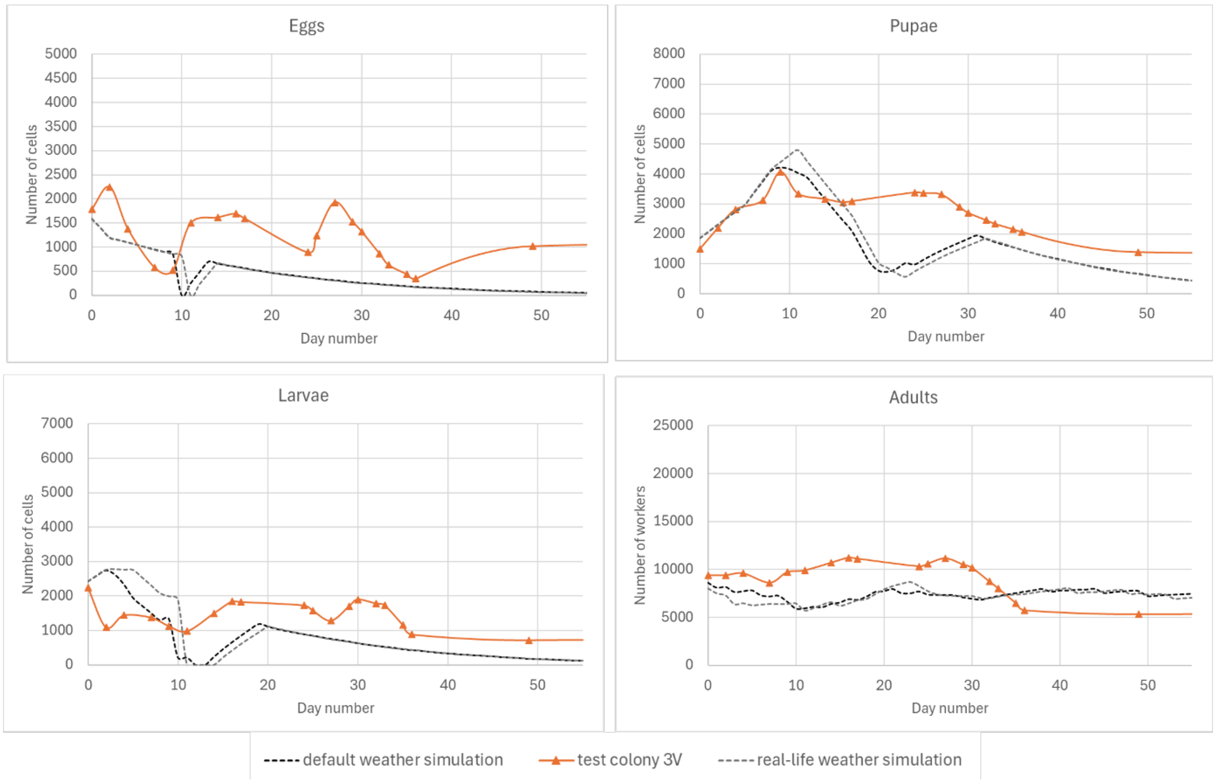


Fig. 37 Low space response sample real-life data and simulation outcomes comparison. (Source: own materials)

As can be seen in Figs 35-37, model's predictions are the most accurate for the pupae stage and the least for the egg stage. In all cases previously observed, an inverse prediction of the number of adults is present as well. Some artifacts are visible for both space scenarios outcomes, not reflected by the real-life data. However, as the amount of available data in the research context is very scarce, an important limitation exists in the interpretation of the results obtained.

6.4.5. Aging consideration

Model prediction for the real-life colony and weather data with age consideration was additionally performed. All the parameters previously included in Chapter 6.1.4 are under further consideration as well.

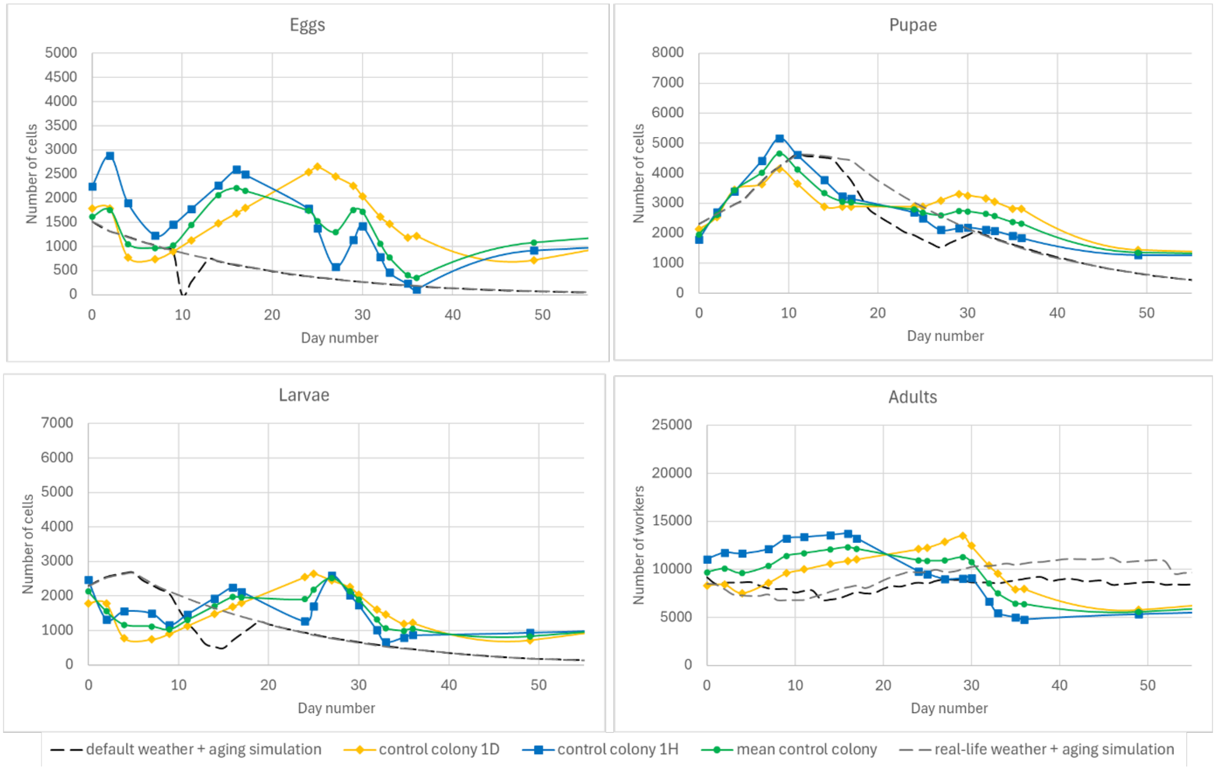


Fig. 38 Comparison of the control sample real-life data and outcomes of the simulation considering queen's aging. (Source: own materials)

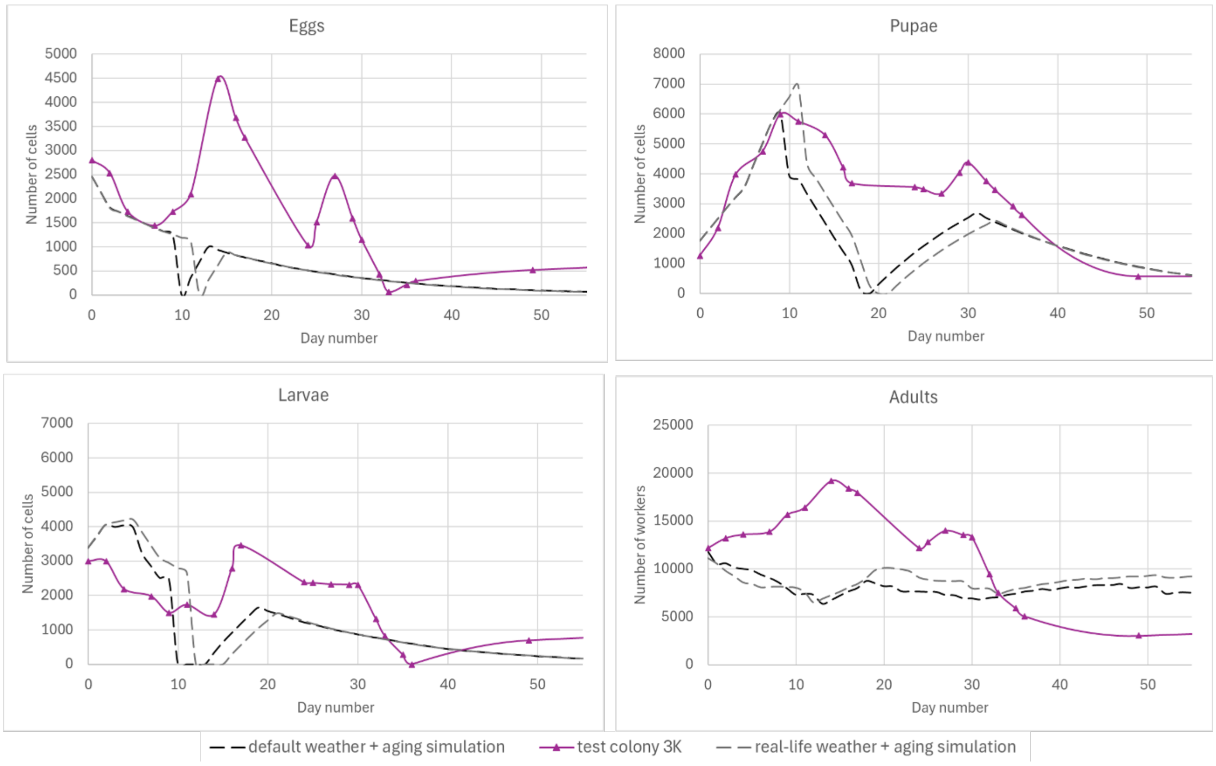


Fig. 39 Comparison of the high response scenario real-life data and outcomes of the simulation considering queen's aging. (Source: own materials)

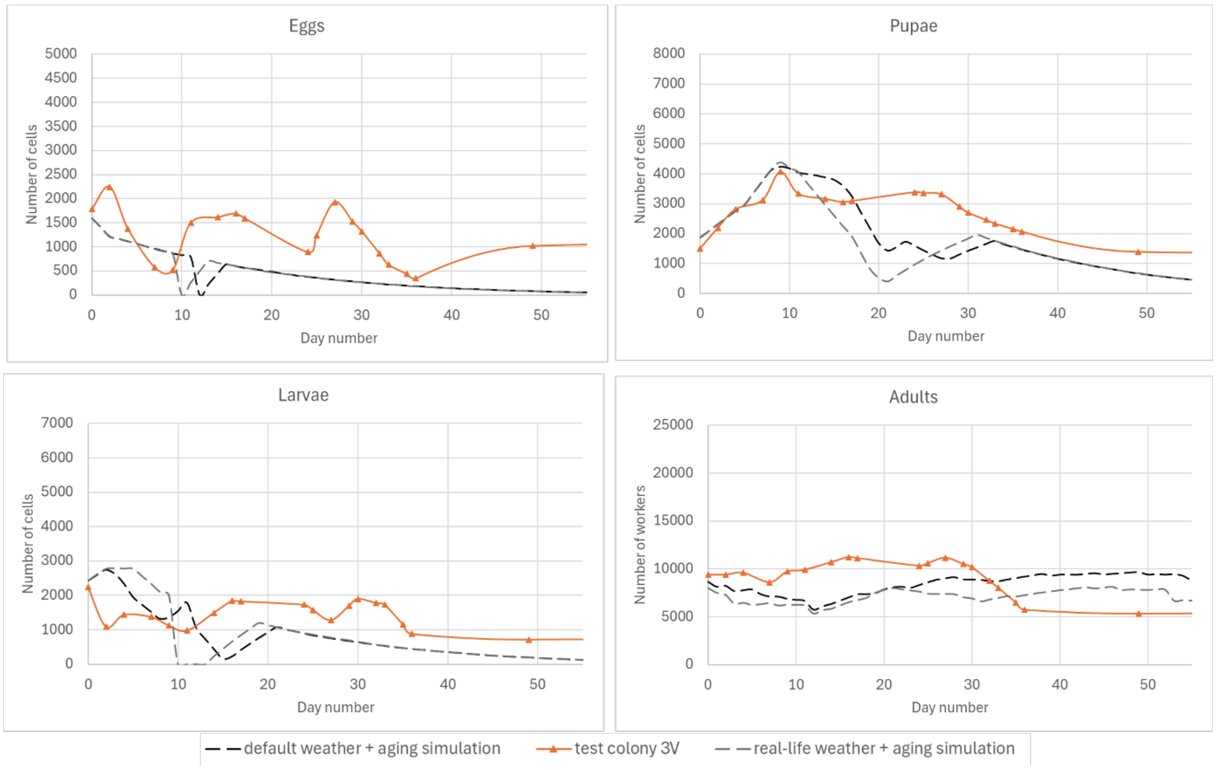


Fig. 40 Comparison of the low response scenario real-life data and outcomes of the simulation considering queen's aging. (Source: own materials)

Additional consideration of the queen's ageing slightly improved the model's predictions in all considered cases (Fig. 38-40). Artifacts are still visible for both space scenarios, except for the low space response pupae, where the pupae number does not drop to zero but only drops significantly for the real-life weather data simulation.

6.4.6. After overwintering development – introduction into new season

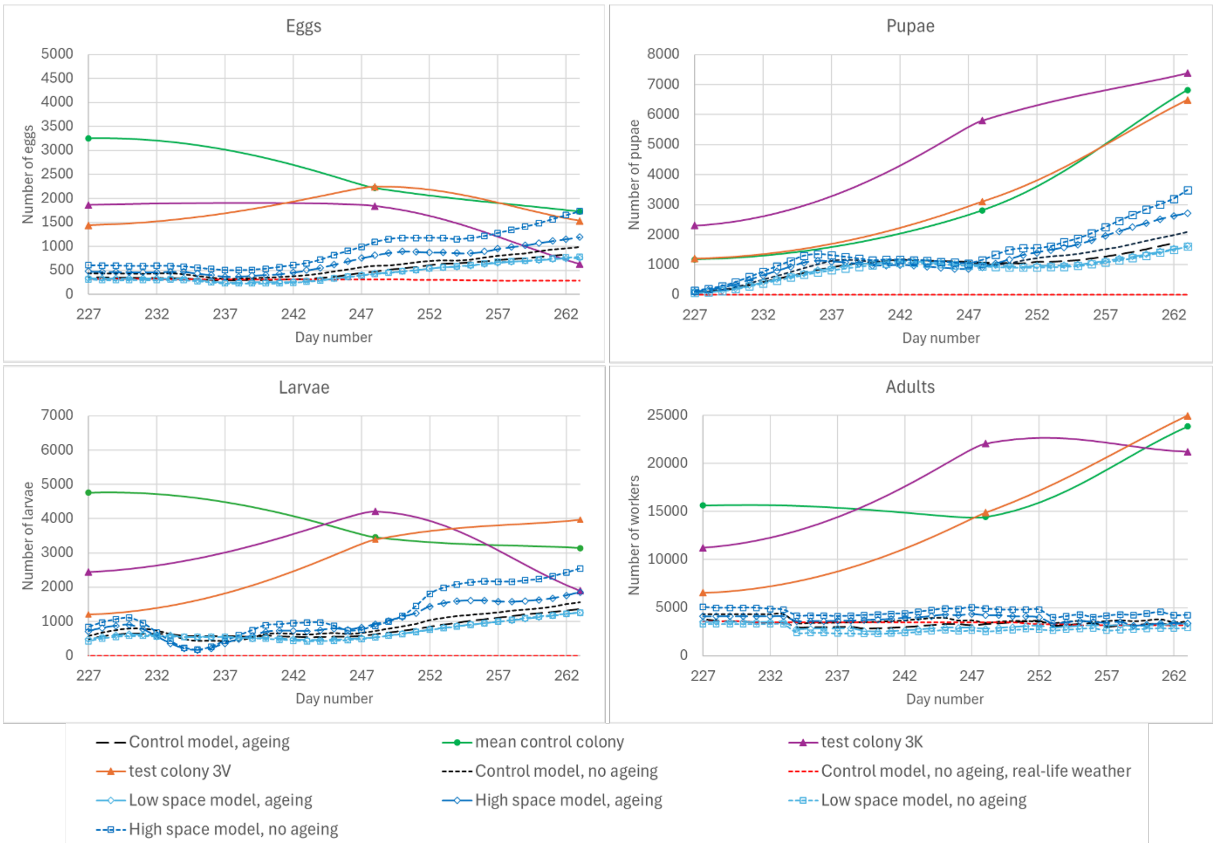


Fig. 41 Comparison of observational data on after-overwintering development with BEEHAVE's predictions, considering various factors (real-life weather data, ageing). (Source: own materials)

Figure 41 presents the after-overwintering period, covered by 3 points of the field data in early spring, as described in Chapter 4. The control model refers to model prepared basing on the mean initial number of brood on the control sample, the low space model refers to a model prepared basing on the initial number of brood in the low space response sample (3V), and the high space model refers to a model prepared basing on initial number of brood in the high space response sample (3K). As can be seen, none of the predictions for entering new season after overwintering was correct in any scenario. None of them correctly predicted the number of castes. However, the trend of the change was reasonably good for larvae and pupae in some of the analyses, mostly referring to the low space response scenario. Such a poor model's performance can be caused by the specific real-life weather conditions, significantly differing from the default weather data used by the model. However, using the real-life weather data for the whole simulation period in almost all cases caused colony death during the overwintering time. The exception was the model based on the control sample's initial conditions with no consideration of the ageing of the queen. Nevertheless, these predictions were not in line with the real-life data, most probably due to harsh real-life weather conditions compared to the model's default.

6.4.7. Real-life read-in data

The model described in Chapter 6.4.4. was additionally equipped with colony composition input data in form of the read-in file. The file contained real-life data from observations of the number of eggs, larvae, pupae, and food stores on specific days. Each simulation was provided with the corresponding number of the mentioned parameters on the simulation starting day. The output of the model considering such input file was then compared to the regular predictions to assess the overall effects of the modification.

Real-life weather data, low space response initial conditions:
exp_input_initial_conditions: On (hive composition defined in Table 6)*; exp_Age-of-Queen:* 56
VarroaTreatment: On; *FeedBees*: On

ReadBeeMappFile: Off

ReadBeeMappFile: On

Fig. 42 Model outputs comparison – the left side presents output of the model for the low space response colony under real-life weather conditions with treatment and feeding included, the right side presents the same model additionally considering read-in assessment file. (Source: BEEHAVE output plots, based on own data)

Default weather data, low space response initial conditions:
exp_input_initial_conditions: On (hive composition defined in Table 6)*; exp_Age-of-Queen:* 56
VarroaTreatment: On; *FeedBees*: On

ReadBeeMappFile: Off

ReadBeeMappFile: On

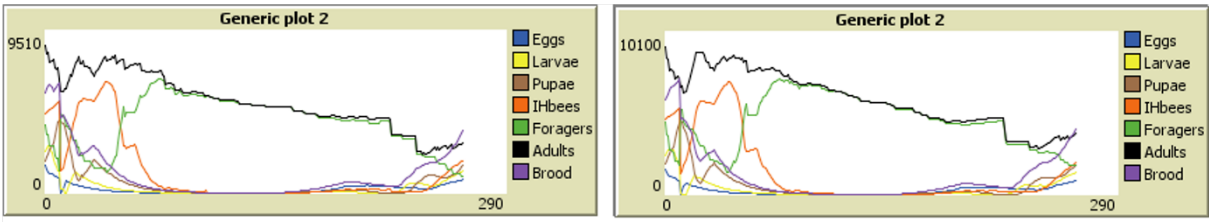
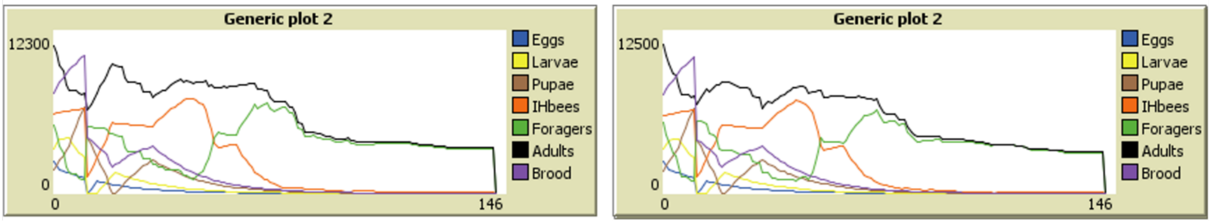


Fig. 43 Model outputs comparison for default weather conditions – the left side considers no read-in assessment file, the right side does consider it. (Source: BEEHAVE output plots, based on own data)

As shown in Fig. 42, the read-in file changes the predicted colony lifespan for the low space response colony. Despite the previously observed negative impact of real-life weather conditions on the model's predictions, predicting colonies to collapse under real-life weather conditions, the combination of real-life weather data and read-in real-life assessment file changes the model's prediction to surviving winter. For the default weather conditions (Fig. 43), both for read-in file and no read-in file scenario, the colony is predicted to survive for the whole simulation time. Additionally, for both real-life (Fig. 42) and default weather conditions (Fig. 43), the colony is predicted to have more adult workers in the read-in file scenarios. That might suggest the model's tendency to underpredict the colony's overall development performance in low space response colonies.

Real-life weather data, high space response initial conditions:
exp_input_initial_conditions: On (hive composition defined in Table 6)*; exp_Age-of-Queen:* 56
VarroaTreatment: On; *FeedBees*: On

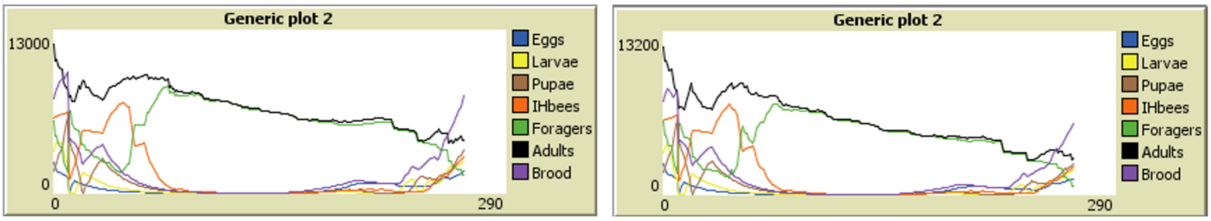


ReadBeeMappFile: Off

ReadBeeMappFile: On

Fig. 44 Model outputs comparison for real-life weather conditions – the left side considers no read-in assessment file, the right side does consider it. No significant differences were observed. (Source: BEEHAVE output plots, based on own data)

Default weather data, high space response initial conditions:
exp_input_initial_conditions: On (hive composition defined in Table 6)*; exp_Age-of-Queen:* 56
VarroaTreatment: On; *FeedBees*: On



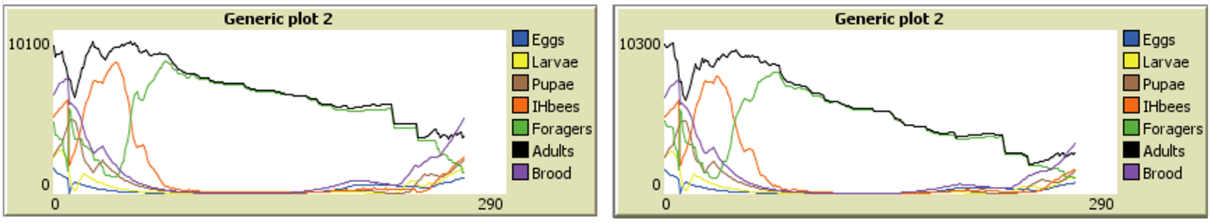
ReadBeeMappFile: Off

ReadBeeMappFile: On

Fig. 45 Model outputs comparison for default weather data – the left side considers no read-in assessment file, the right side considers additional read-in assessment file for the high-response scenario. (Source: BEEHAVE output plots, based on own data)

For the high space response scenario, both analyzed cases (with/without read-in file) predict the colony’s winter collapse under real-life weather data (Fig. 44) and winter survival under default weather conditions (Fig. 45). No significant changes in the maximum number of adults or brood were observed nor in the overall development dynamics.

Default weather data, mean control initial conditions:
exp_input_initial_conditions: On (hive composition defined in Table 6)*; exp_Age-of-Queen:* 56
VarroaTreatment: On; *FeedBees*: On



ReadBeeMappFile: Off

ReadBeeMappFile: On

Fig. 46 Model outputs comparison for default weather conditions – the left side considers no read-in file inclusion, the right side additionally considers read-in assessment file. (Source: BEEHAVE output plots, based on own data)

Real-life weather data, mean control initial conditions:
exp_input_initial_conditions: On (hive composition defined in Table 6)*; exp_Age-of-Queen:* 56
VarroaTreatment: On; *FeedBees*: On

ReadBeeMappFile: Off | *ReadBeeMappFile*: On

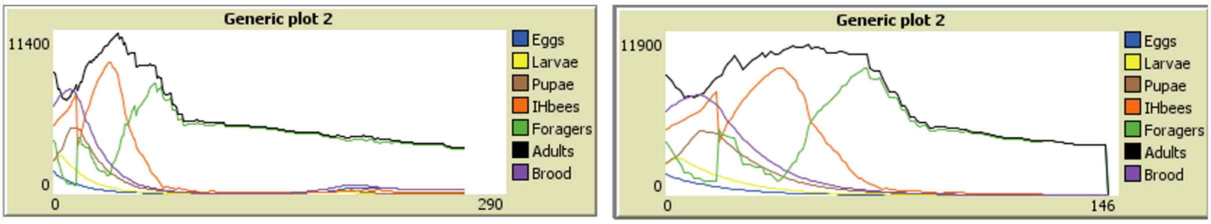


Fig. 47 Model outputs comparison for real-life weather data –the right side presents output for model considering read-in assessment file. (Source: BEEHAVE output plots, based on own data)

In the control scenarios based on the mean control sample data while considering default weather data both read-in file and no read-in file predictions are of similar dynamics (Fig. 46). However, for the real-life weather scenario, model considering read-in colony composition file predicts the colony to collapse during overwintering period (Fig. 47). This is the exact opposite model's reaction in comparison to high space response scenario, where adding real-life data on the colony composition caused changing the model's prediction from winter collapse to winter survival.

Real-life weather data, colony 1H initial conditions:
exp_input_initial_conditions: On (hive composition defined in Table 6)*; exp_Age-of-Queen:* 56
VarroaTreatment: On; *FeedBees*: On

ReadBeeMappFile: Off | *ReadBeeMappFile*: On

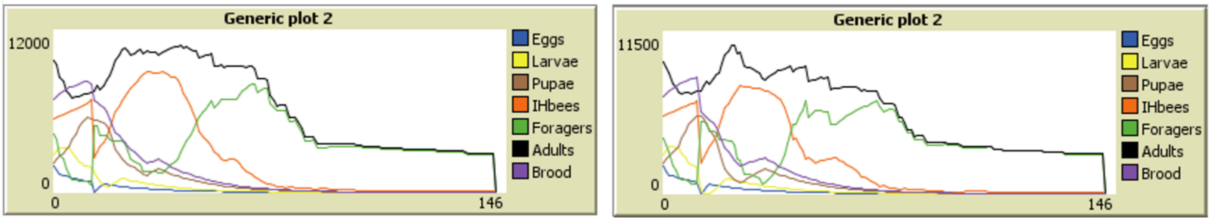


Fig. 48 Model outputs comparison for real-life weather data – the right side presents output of analogous model as the left side, but additionally considering read-in assessment file. (Source: BEEHAVE output plots, based on own data)

Real-life weather data, colony 1D initial conditions:
exp_input_initial_conditions: On (hive composition defined in Table 6)*; exp_Age-of-Queen:* 56
VarroaTreatment: On; *FeedBees*: On

ReadBeeMappFile: Off | *ReadBeeMappFile*: On

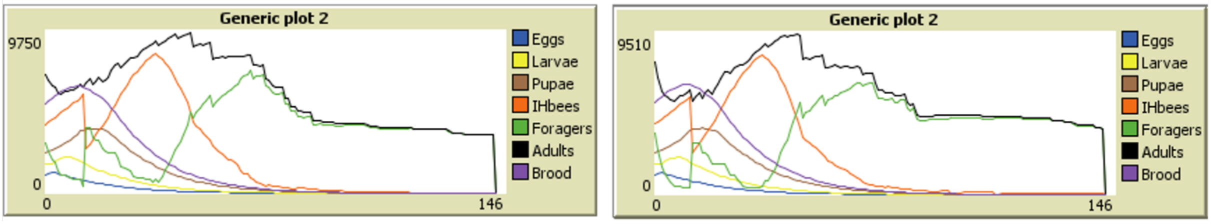


Fig. 49 Model outputs comparison – the left side presents output of the model for the control sample colony 1D with real-life weather conditions, considering treatment and feeding included, the right side presents the same model additionally equipped with read-in assessment file. (Source: BEEHAVE output plots, based on own data)

In this case, the model's behavior can be explained by the data behind the input data under consideration – as can be seen in Figs. 48 and 49, winter collapse was predicted in both scenarios using separate input data from control colonies 1H and 1D, regardless of which weather data was used.

6.5. Model’s sensitivity analysis

Sensitivity analyses were done for the high space response, low space response, and control sample scenarios for each parameter separately. Analysis was performed in an analogous manner to the basic model’s analysis proposed by its authors [73], with a few minor modifications. Such an approach was chosen due to the quality and satisfying range of the proposed method, and all the inconsistencies were dictated by the researched context specifics. Additionally, apart from the output of colony size after three years, colony size after one year was analyzed for the same rationale.

During the analysis, each single parameter was multiplied by a factor ranging from 0.1 to 4 (x-axis in Figures 50-105). Default values (for factor equal 1) are marked on graphs as white circles; all the other values are marked with black dots. In total, 28 parameters were analyzed. Such a large range of parameter variation provides a comprehensive overview of single parameter effects on model behavior.

Performed analyses are presented in the following sections, separately for the parameters affecting model behavior strongly and to a limited extent and separately for parameters causing no significant variation in the model’s behavior. Sensitivity of ten parameters was relatively high, indicating possible strong correlation of the parameters with colony size.

Part of the parameters considered by the model, such as mite infestation or swarming, were excluded from the sensitivity analysis due to low or very low probability of occurrence in extraterrestrial greenhouse conditions where queen bee transported by the rocket will be functioning.

6.5.1. Parameters visibly affecting model behavior

6.5.1.1. Volume of a forager's honey stomach (CROPVOLUME)

Default value: 50 µl

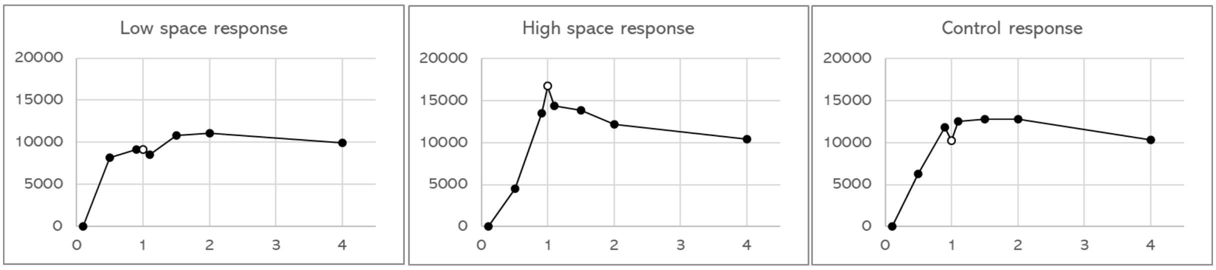


Fig. 50 Colony size after one year sensitivity to the volume of a forager's crop (CROPVOLUME) parameter changes for low space response, high space response and control response scenarios.

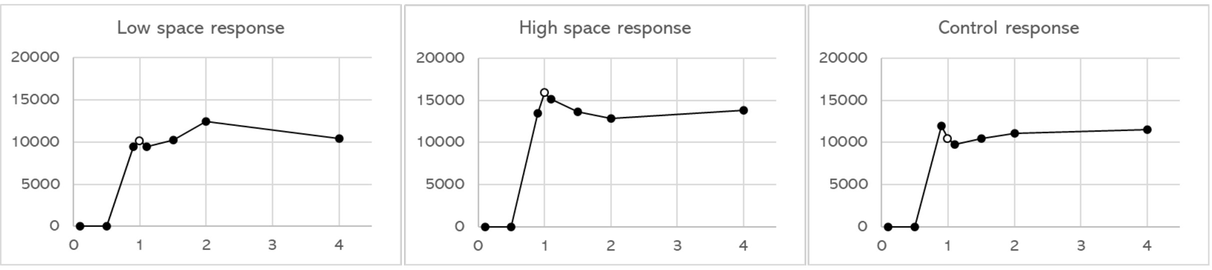


Fig. 51 Colony size after three years sensitivity to the volume of a forager's crop (CROPVOLUME) parameter changes for low space response, high space response and control response scenarios.

The parameter specifies the volume of a forager's honey stomach (crop) and is considered rather constant based on the experimental data. As can be seen in Fig. 50 and 51, the model is sensitive to its changes, especially when the volume drops below 90% of the original value (50 µl). The model's sensitivity can be an indication to verify the parameter for workers born in extraterrestrial conditions and/or developed from eggs laid by the queen, which was given to the space travel/hypergravity conditions.

6.5.1.2. Maximum lifespan of a worker bee (LIFESPAN)

Default value: 290 days

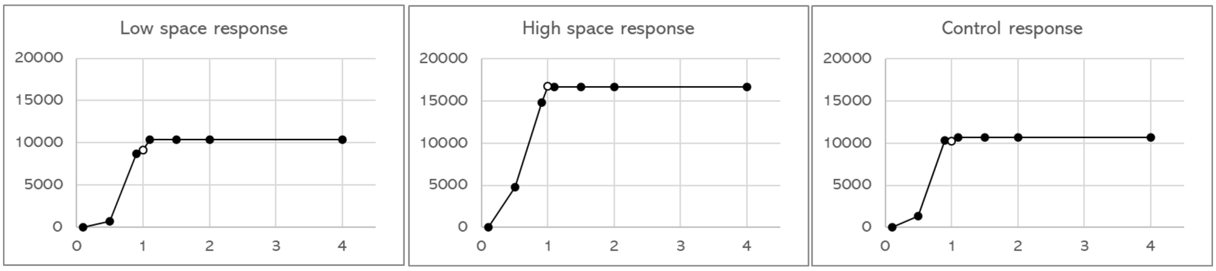


Fig. 52 Colony size after one year sensitivity to the maximum lifespan of a worker bee (LIFESPAN) parameter changes for low space response, high space response and control response scenarios.

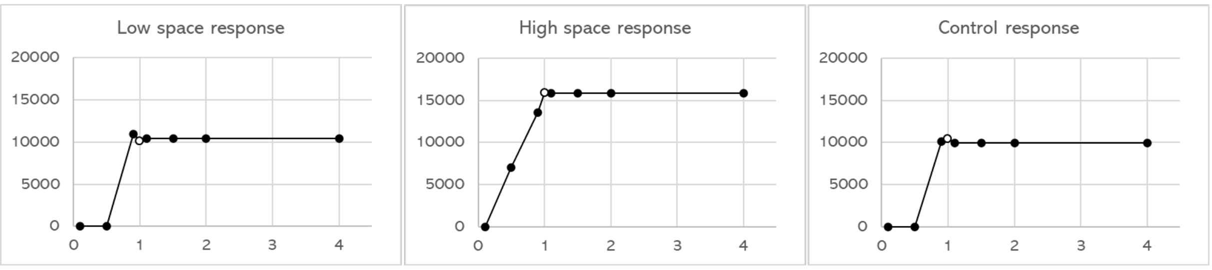


Fig. 53 Colony size after three years sensitivity to the maximum lifespan of a worker bee (LIFESPAN) parameter changes for low space response, high space response and control response scenarios.

The maximum lifespan of a worker bee has no significant impact on the model when it's greater than the original value (290 days). However, for smaller values, the model's sensitivity increases significantly, and in extreme cases, the colony collapse is predicted. Similarly to the CROPVOLUME parameter, worker bee maximum lifespan in extraterrestrial conditions shall be then verified.

6.5.1.3. Maximum amount of brood, nurse bees can care for (MAX_BROOD_NURSE_RATIO)

Default value: 3

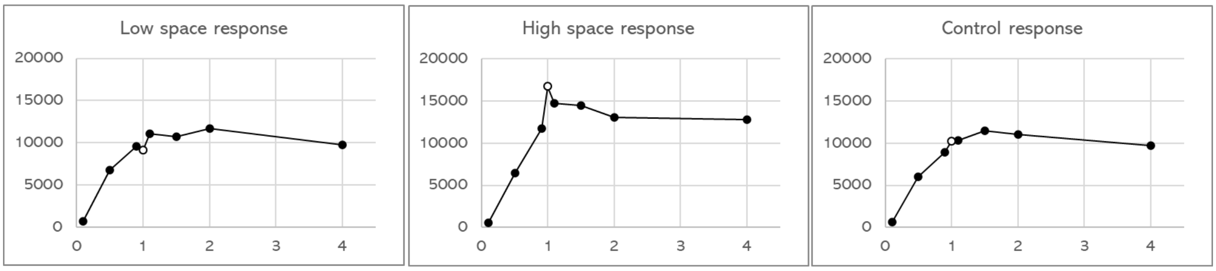


Fig. 54 Colony size after one year sensitivity to the maximum amount of brood, nurse bees can care for (MAX_BROOD_NURSE_RATIO) parameter changes for low space response, high space response and control response scenarios.

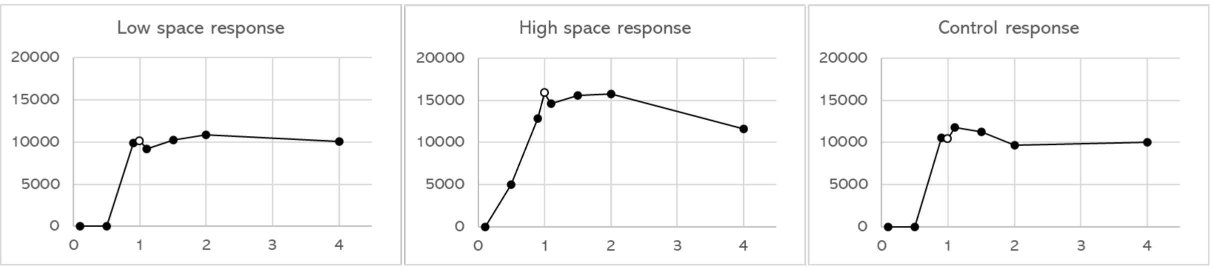


Fig. 55 Colony size after three years sensitivity to the maximum amount of brood, nurse bees can care for (MAX_BROOD_NURSE_RATIO) parameter changes for low space response, high space response and control response scenarios.

In general, the model is sensitive to the parameter's value lower than the original. However, in the case of a high space response scenario, the maximum amount of brood a nurse bee can care for affects the colony's overall performance negatively, also for values greater than the original.

6.5.1.4. Maximum egg laying rate per day (MAX_EGG_LAYING)

Default value: 1,600 eggs/day

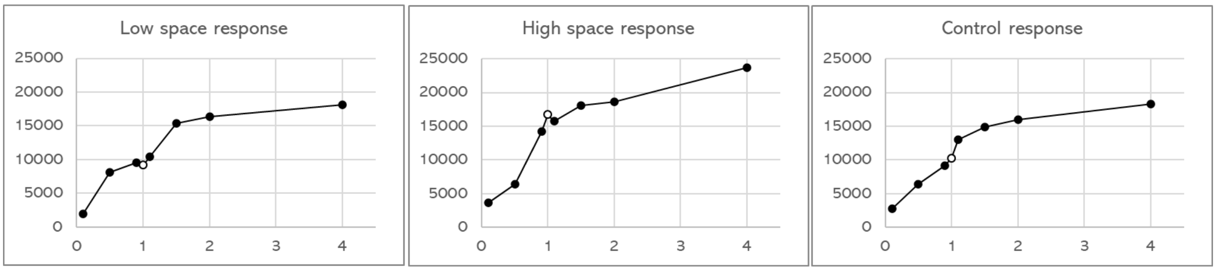


Fig. 56 Colony size after one year sensitivity to the maximum egg laying rate per day (MAX_EGG_LAYING) parameter changes for low space response, high space response and control response scenarios.

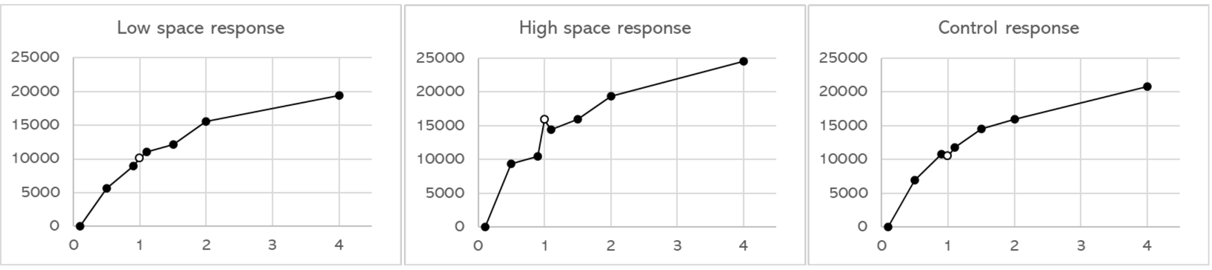


Fig. 57 Colony size after three years sensitivity to the maximum egg laying rate per day (MAX_EGG_LAYING) parameter changes for low space response, high space response and control response scenarios.

The model is sensitive to the queen's maximum egg-laying rate per day. The observed tendency is in line with the beekeepers' observations, indicating that the higher the egg-laying rate, the bigger the colony.

6.5.1.5. Maximum honey amount that can be stored (MAX_HONEY_STORE_kg)

Default value: 50 kg

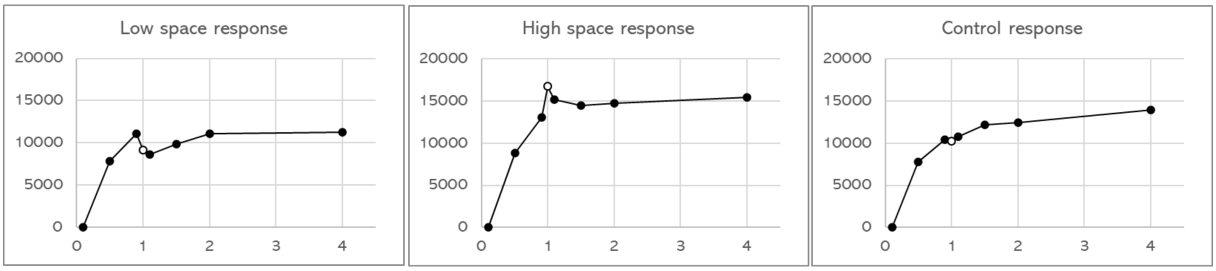


Fig. 58 Colony size after one year sensitivity to the maximum honey amount that can be stored (MAX_HONEY_STORE_kg) parameter changes for low space response, high space response and control response scenarios.

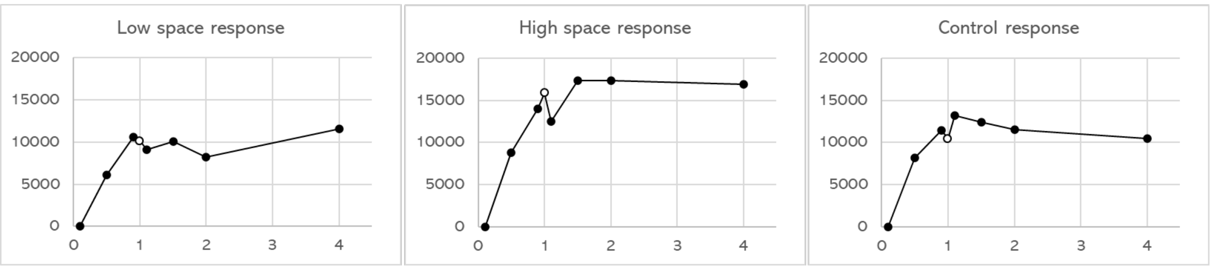


Fig. 59 Colony size after three years sensitivity to the maximum honey amount that can be stored (MAX_HONEY_STORE_kg) parameter changes for low space response, high space response and control response scenarios.

Model shows the greatest sensitivity within maximum honey weight below 90% of the original value (50 kg). The parameter is particularly important in the context of using various hive types, especially smaller models, such as the mini-plus used in the experiment from the Chapter 4. Interestingly, for three years long scenario for control sample mass of honey stores exceeding 50 kg also affects the colony negatively.

6.5.1.6. Mortality rate of foragers per second of foraging (MORTALITY_FOR_PER_SEC)

Default value: 0.00001 mortality/s

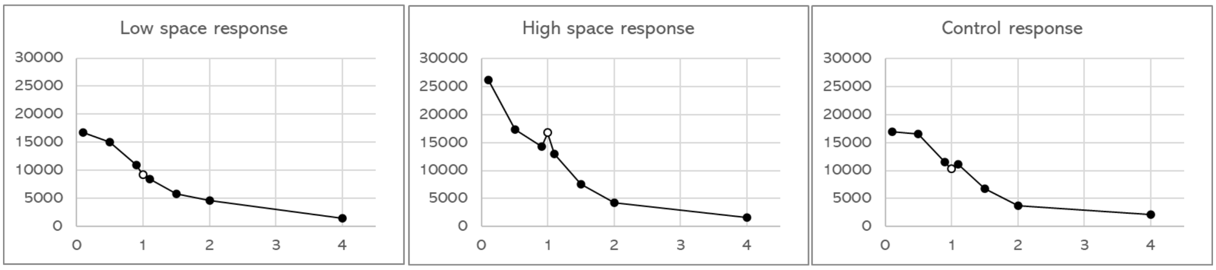


Fig. 60 Colony size after one year sensitivity to the mortality rate of foragers per second of foraging (MAX_FOR_PER_SEC) parameter changes for low space response, high space response and control response scenarios.

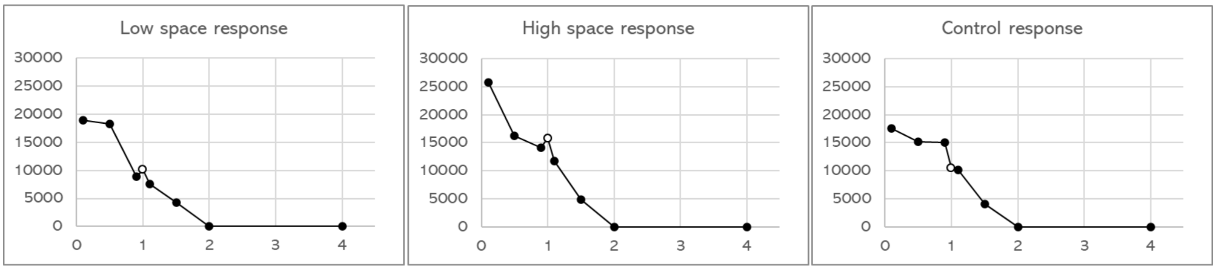


Fig. 61 Colony size after three years sensitivity to the mortality rate of foragers per second of foraging (MAX_FOR_PER_SEC) parameter changes for low space response, high space response and control response scenarios.

The model is sensitive to the mortality rate of foragers per second of foraging as expected. For three years long analysis it is lethal to modeled colonies already after doubling the original value.

6.5.1.7. Initial colony size (N_INITIAL_BEES)

Default value: 10,000 bees

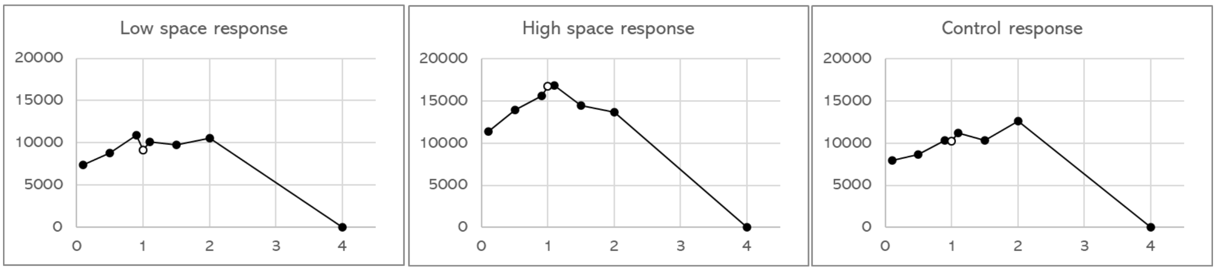


Fig. 62 Colony size after one year sensitivity to the initial colony size (N_INITIAL_BEES) parameter changes for low space response, high space response and control response scenarios.

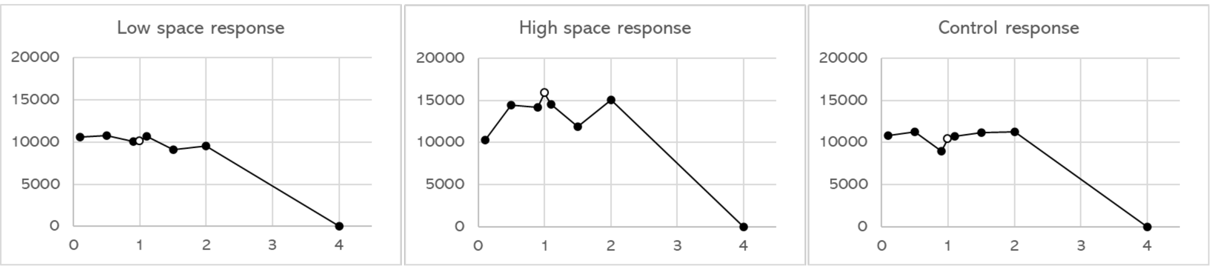


Fig. 63 Colony size after three years sensitivity to the initial colony size (N_INITIAL_BEES) parameter changes for low space response, high space response and control response scenarios.

Initial colony size affects the model the most in case of multiplication of the default value by four, leading colonies to death.

6.5.1.8. Amount of pollen collected during single, successful foraging trip (POLLENLOAD)

Default value: 0.015 g

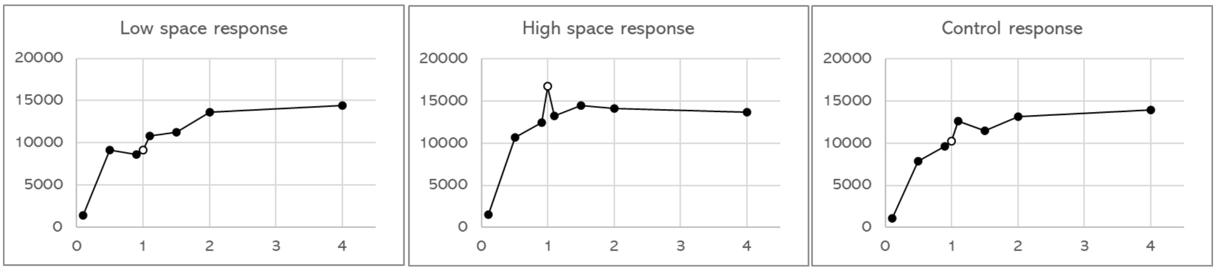


Fig. 64 Colony size after one year sensitivity to the amount of pollen collected during single, successful foraging trip (POLLENLOAD) parameter changes for low space response, high space response and control response scenarios.

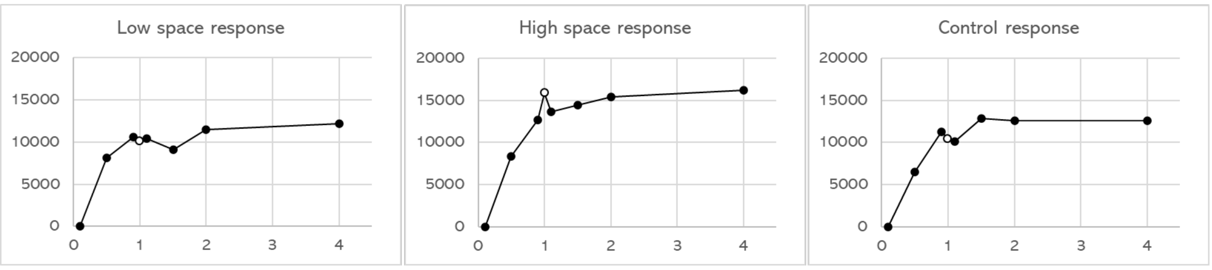


Fig. 65 Colony size after one year sensitivity to the amount of pollen collected during single, successful foraging trip (POLLENLOAD) parameter changes for low space response, high space response and control response scenarios.

The parameter represents the amount of pollen collected during a single, successful pollen foraging trip. Model is particularly sensitive to lowering the original value, which may be particularly important in case of extraterrestrial crops where the value might be influenced by, e.g., altered gravity conditions or flawed design of the greenhouse.

6.5.1.9. Time to fill honey stomach with nectar (TIME_NECTAR_GATHERING)

Default value: 1,200 s

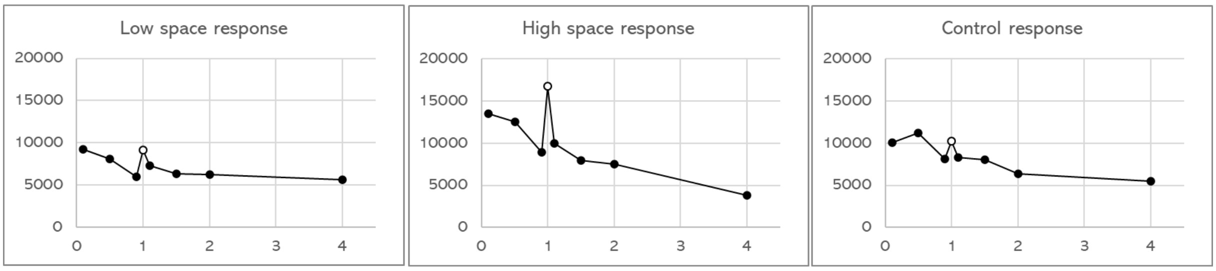


Fig. 66 Colony size after one year sensitivity to the time to fill honey stomach with nectar (TIME_NECTAR_GATHERING) parameter changes for low space response, high space response and control response scenarios.

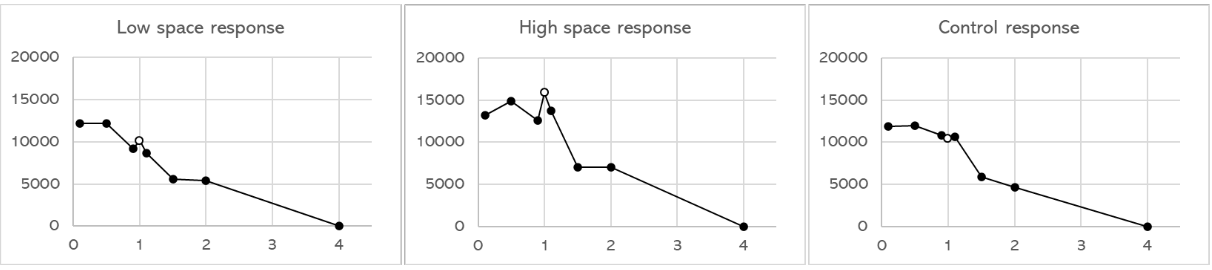


Fig. 67 Colony size after three years sensitivity to the time to fill honey stomach with nectar (TIME_NECTAR_GATHERING) parameter changes for low space response, high space response and control response scenarios.

Parameter refers only to situation when the general nectar quantity was not yet reduced in the flower patch. The model is more sensitive to the parameter the longer is the simulation time. Moreover, for the 3-years-long observation significant increase in the parameter's value may lead to colony collapse. For 1-year analysis for high space response scenario any divergence from the original value causes a drop in the colony size. As the parameter refers to the efficiency of foragers, it highlights the importance of the research on the altered gravity impact on foraging.

6.5.1.10. Time to collect a pollen load (TIME_POLLEN_GATHERING)

Default value: 600 s

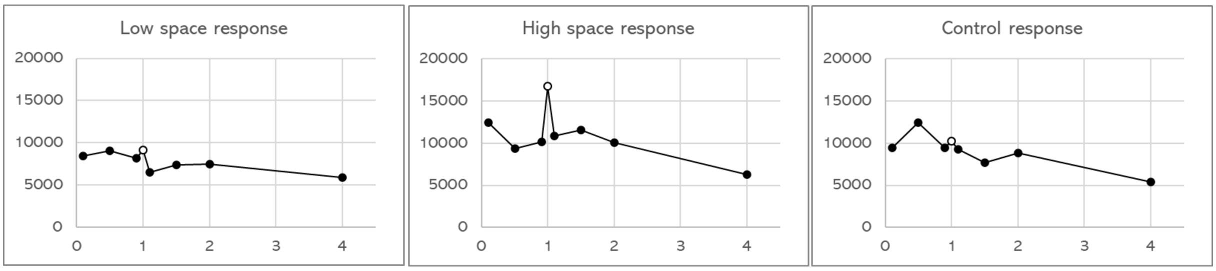


Fig. 68 Colony size after one year sensitivity to the time to collect a pollen load (TIME_POLLEN_GATHERING) parameter changes for low space response, high space response and control response scenarios.

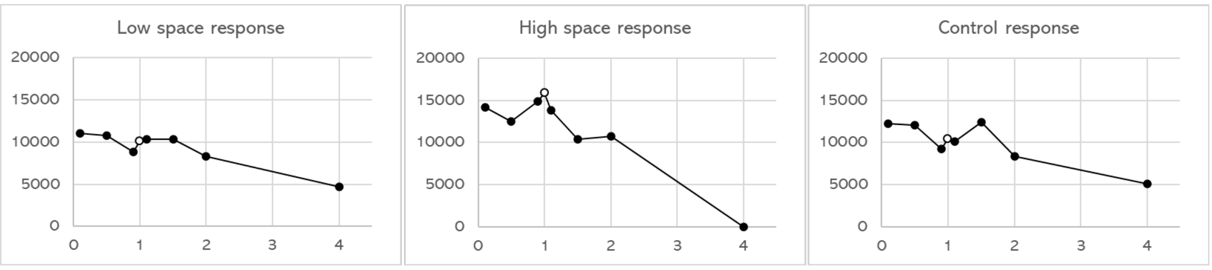


Fig. 69 Colony size after three years sensitivity to the time to collect a pollen load (TIME_POLLEN_GATHERING) parameter changes for low space response, high space response and control response scenarios.

The parameter refers to the situation when pollen quantity in the flower patch was not yet reduced, exclusively. The longer simulation time is considered, the more the model is sensitive to the parameter. The most sensitive is high space response colony, while low space response shows the greatest stability of predictions. Similarly to the previous parameter, model's sensitivity to the parameter highlights the importance of both the research on the altered gravity impact on foraging capabilities of worker bees and sufficient crop size and diversity in terms of pollen recovery.

6.5.2. Parameters affecting the model to the limited extent

6.5.2.1. Foragers probability to abandon current pollen patch (ABANDON_POLLEN_PATCH_PROB_PER_S)

Default value: 0.00002/s

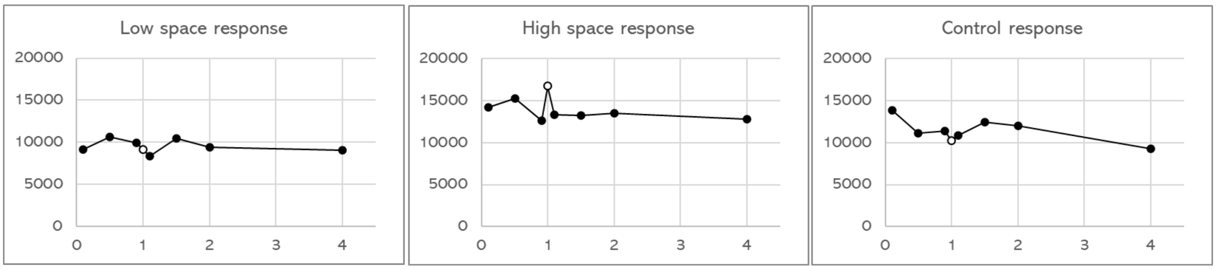


Fig. 70 Colony size after one year sensitivity to the foragers probability to abandon current pollen patch (ABANDON_POLLEN_PATCH_PROB_PER_s) parameter changes for low space response, high space response and control response scenarios.

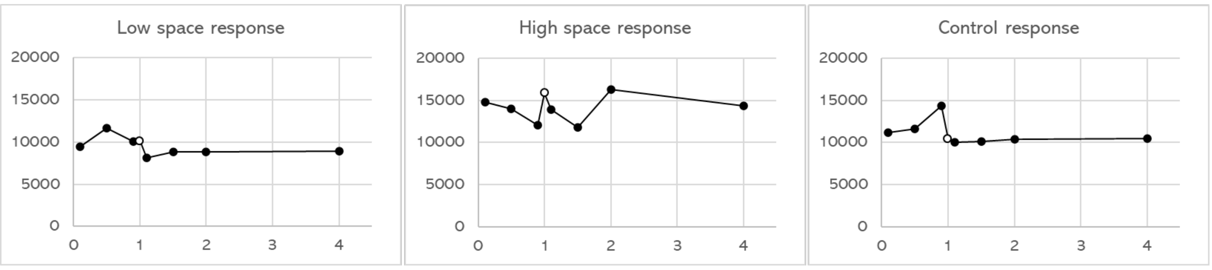


Fig. 71 Colony size after three years sensitivity to the foragers probability to abandon current pollen patch (ABANDON_POLLEN_PATCH_PROB_PER_s) parameter changes for low space response, high space response and control response scenarios.

The greatest disturbances are visible for variations in the parameter in range of 0.5-1.5.

6.5.2.2. Age of first foraging (AFF_BASE)

Default value: 21 days

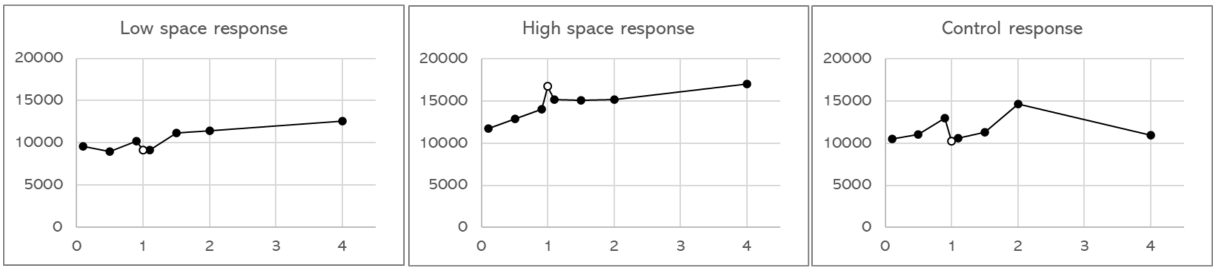


Fig. 72 Colony size after one year sensitivity to the age of first foraging (AFF_BASE) parameter changes for low space response, high space response and control response scenarios.

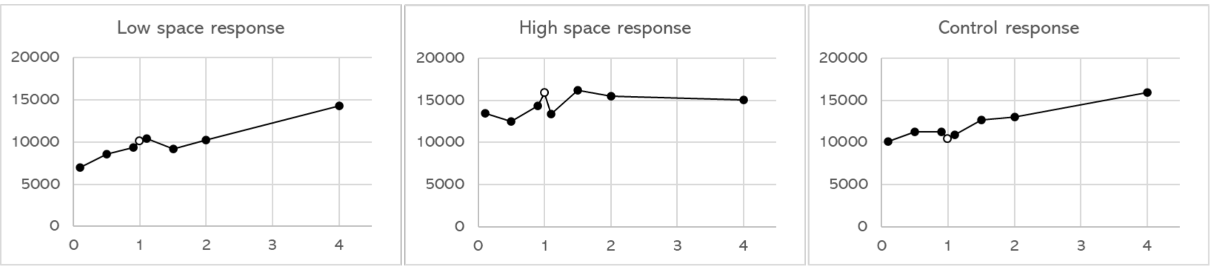


Fig. 73 Colony size after three years sensitivity to the age of first foraging (AFF_BASE) parameter changes for low space response, high space response and control response scenarios.

Age of fist foraging impacts the model to the limited extent. Some possible disturbances are visible for minor value changes.

6.5.2.3. Threshold colony size for winter survival (CRITICAL_COLONY_SIZE_WINTER)

Default value: 4,000 bees

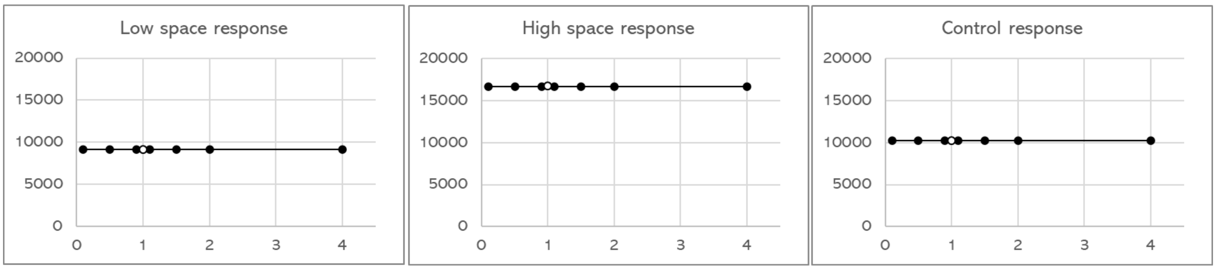


Fig. 74 Colony size after one year sensitivity to the threshold colony size for winter survival (CRITICAL_COLONY_SIZE_WINTER) parameter changes for low space response, high space response and control response scenarios.

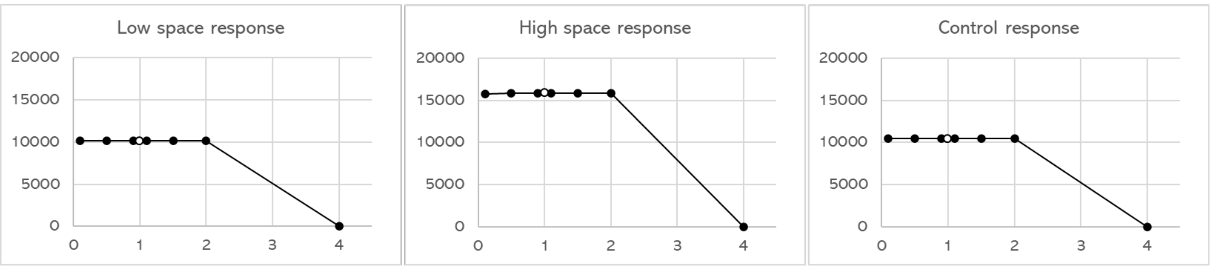


Fig. 75 Colony size after three years sensitivity to the threshold colony size for winter survival (CRITICAL_COLONY_SIZE_WINTER) parameter changes for low space response, high space response and control response scenarios.

The parameter is being checked by the model on day 365 of the simulation. Critical colony size does not affect the model in short time simulations. However, for 3-year-long simulation runs, for parameter being four times the default, the model predicts colonies to die in all the considered scenarios.

6.5.2.4. Maximum lifespan of a drone (DRONE_LIFESPAN)

Default value: 37 days

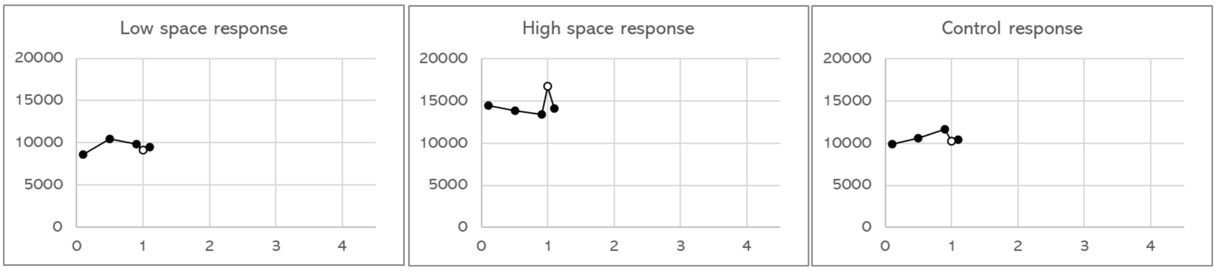


Fig. 76 Colony size after one year sensitivity to the maximum lifespan of a drone (DRONE_LIFESPAN) parameter changes for low space response, high space response and control response scenarios.

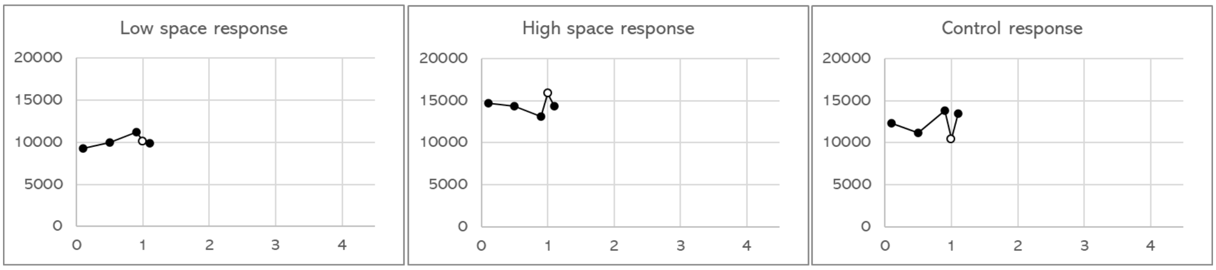


Fig. 77 Colony size after three years sensitivity to the maximum lifespan of a drone (DRONE_LIFESPAN) parameter changes for low space response, high space response and control response scenarios.

For the parameter multiplied by the number exceeding 1.1, code error occurred, so no scenario for drones outliving 40 days of age could have been verified.

6.5.2.5. Maximum brood space (MAX_BROODCELLS)

The default value of the maximum number of cells available for the brood is 2,000,099, while the value calculated for the hive type used in the previously described experiment (mini-plus hive) is 55,200. For that reason, as a default parameter value, 100,000 cells were considered.

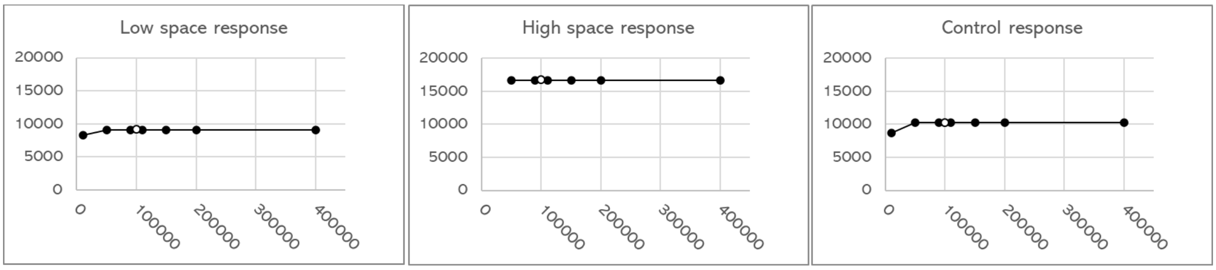


Fig. 78 Colony size after one year sensitivity to the maximum brood space (MAX_BROODCELLS) parameter changes for low space response, high space response and control response scenarios.

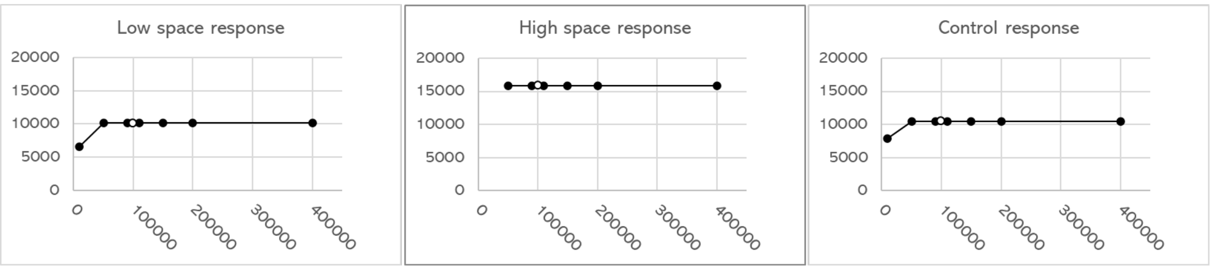


Fig. 79 Colony size after three years sensitivity to the maximum brood space (MAX_BROODCELLS) parameter changes for low space response, high space response and control response scenarios.

In both cases, for maximum brood cells number equal 10,000 for the high space response scenario, an execution error occurred in the model. For three a 3-year-long simulation for maximally 10,000 brood cells available, both low space response and control response scenarios showed a slight drop in the predicted colony size.

6.5.2.6. Daily mortality rate of drone eggs (MORTALITY_DRONE_EGGS)

Default value: 0.064 mortality/day

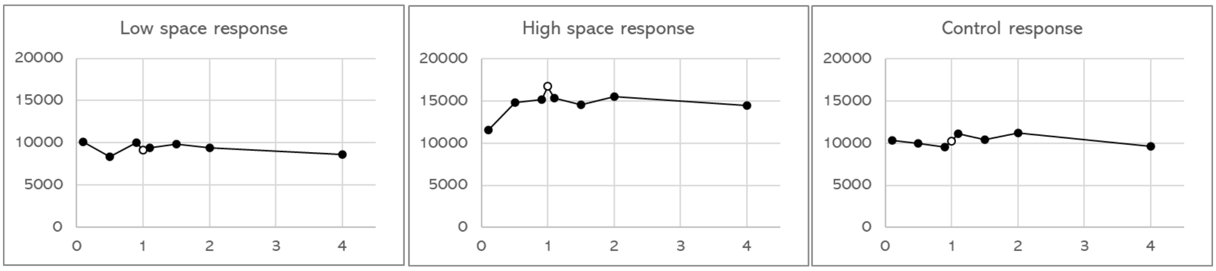


Fig. 80 Colony size after one year sensitivity to the daily drone eggs mortality (MORTALITY_DRONE_EGGS) parameter changes for low space response, high space response and control response scenarios.

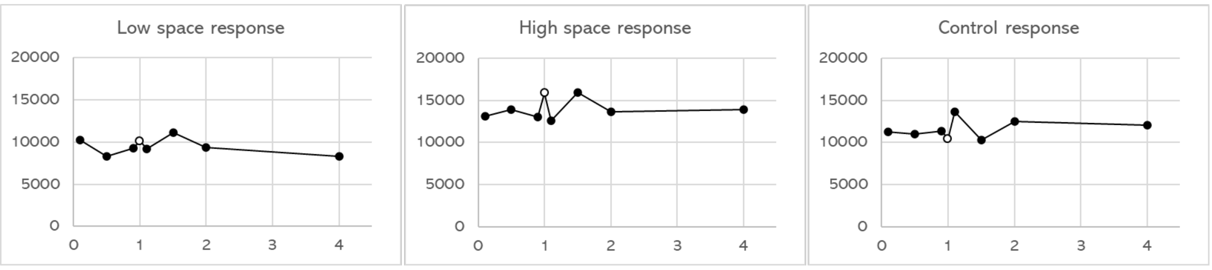


Fig. 81 Colony size after three years sensitivity to the daily drone eggs mortality (MORTALITY_DRONE_EGGS) parameter changes for low space response, high space response and control response scenarios.

In a one-year simulation, a high space response scenario significantly lowers the mortality of drone eggs and lowers the general colony size.

6.5.2.7. Daily mortality of rate of worker eggs (MORTALITY_EGGS)

Default value: 0.03 mortality/day

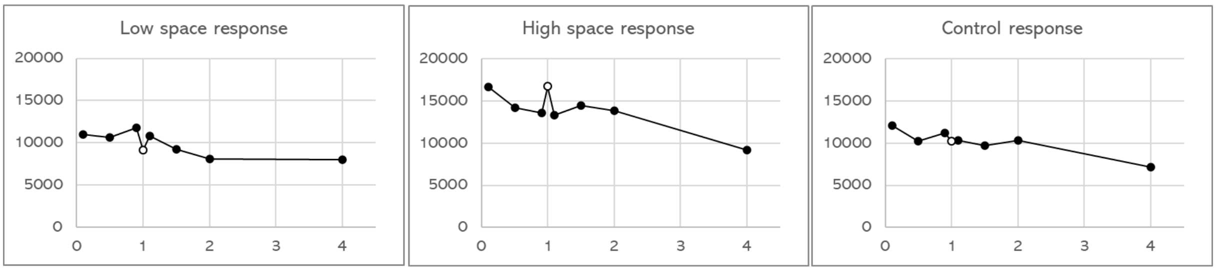


Fig. 82 Colony size after one year sensitivity to the daily worker eggs mortality (MORTALITY_EGGS) parameter changes for low space response, high space response and control response scenarios.

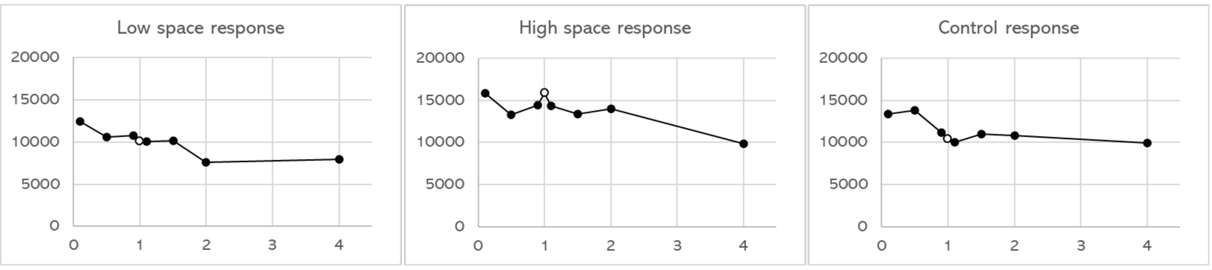


Fig. 83 Colony size after three years sensitivity to the daily worker eggs mortality (MORTALITY_EGGS) parameter changes for low space response, high space response and control response scenarios.

The model is sensitive to increased egg mortality, especially in high space response and control response scenarios.

6.5.2.8. Time to unload pollen in the colony (TIME_UNLOADING_POLLEN)

Default value: 210 s

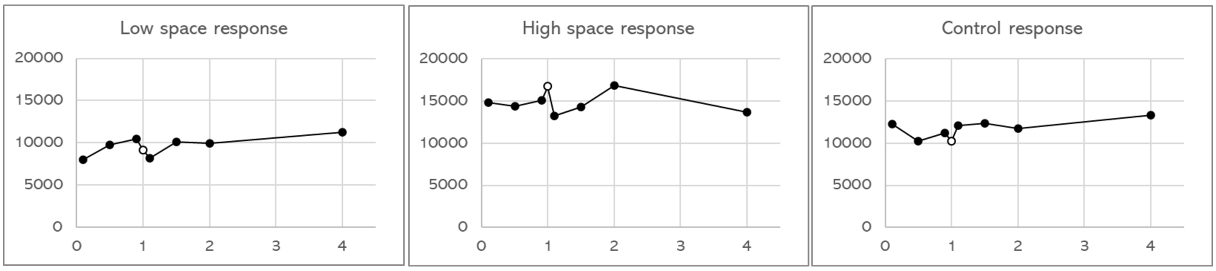


Fig. 84 Colony size after one year sensitivity to the time to unload pollen (TIME_UNLOADING_POLLEN) parameter changes for low space response, high space response and control response scenarios.

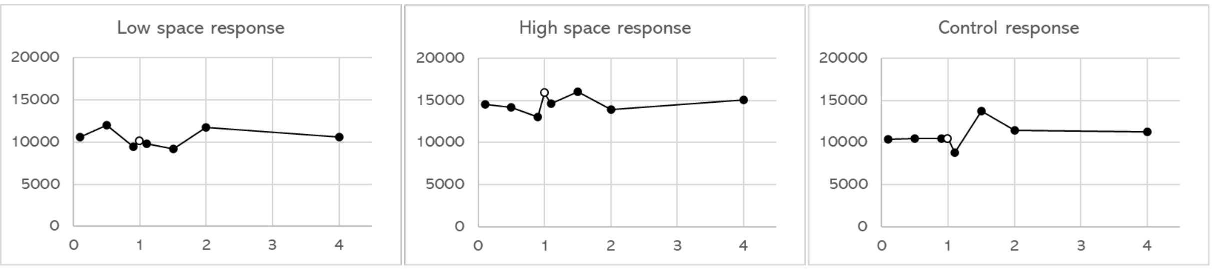


Fig. 85 Colony size after three years sensitivity to the time to unload pollen (TIME_UNLOADING_POLLEN) parameter changes for low space response, high space response and control response scenarios.

The model shows the greatest sensitivity to the time required for pollen unloading for a change factor ranging from 0.1 to 2. As the value might be impacted by the altered gravity conditions, it should be verified if any change in bees' behavior appears when working under altered gravity conditions.

6.5.3. Parameters with no significant impact on model behavior

6.5.3.1. First day of drone production (DRONE_EGGLAYING_START)

Default value: day 115

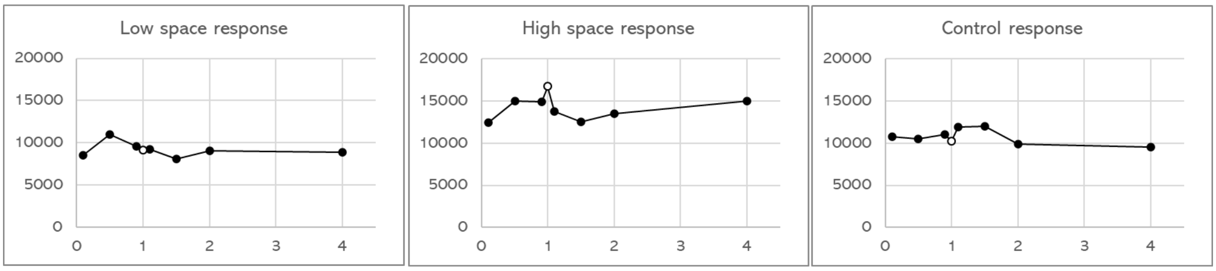


Fig. 86 Colony size after one-year sensitivity to the beginning of drone production (DRONE_EGGLAYING_START) parameter changes for low space response, high space response and control response scenarios.

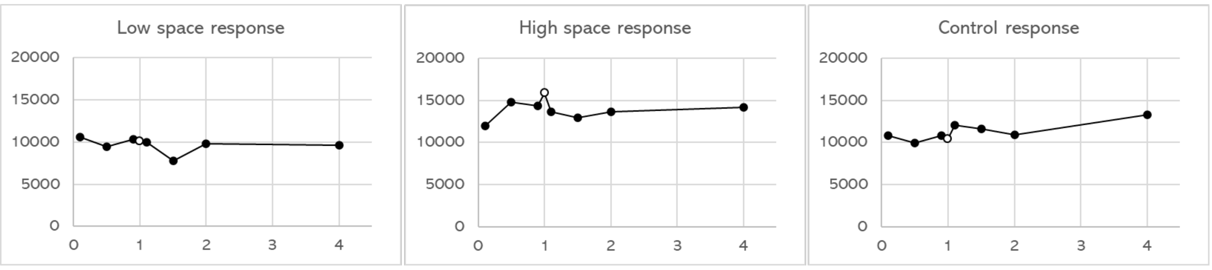


Fig. 87 Colony size after three years sensitivity to the beginning of drone production (DRONE_EGGLAYING_START) parameter changes for low space response, high space response and control response scenarios.

6.5.3.2. Last day of drone production (DRONE_EGGLAYING_STOP)

Default value: day 240

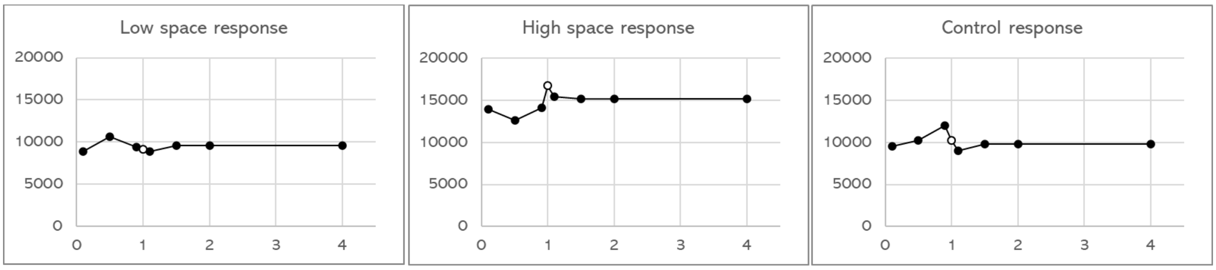


Fig. 88 Colony size after one-year sensitivity to the end of drone production (DRONE_EGGLAYING_STOP) parameter changes for low space response, high space response and control response scenarios.

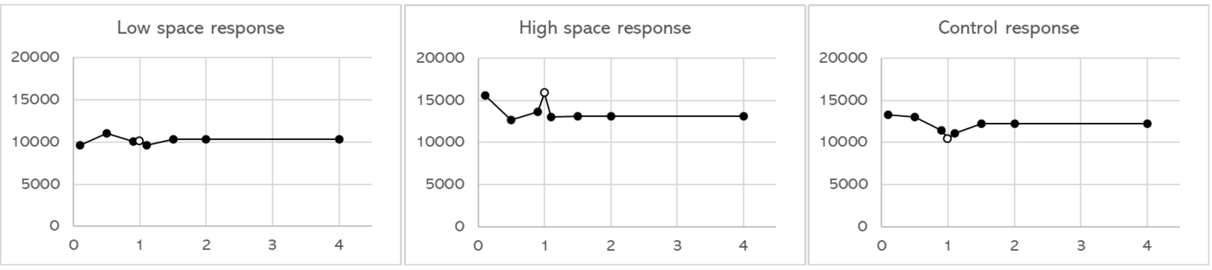


Fig. 89 Colony size after three years sensitivity to the end of drone production (DRONE_EGGLAYING_STOP) parameter changes for low space response, high space response and control response scenarios.

6.5.3.3. Proportion of drone eggs (DRONE_EGGS_PROPORTION)

Default value: 0.04

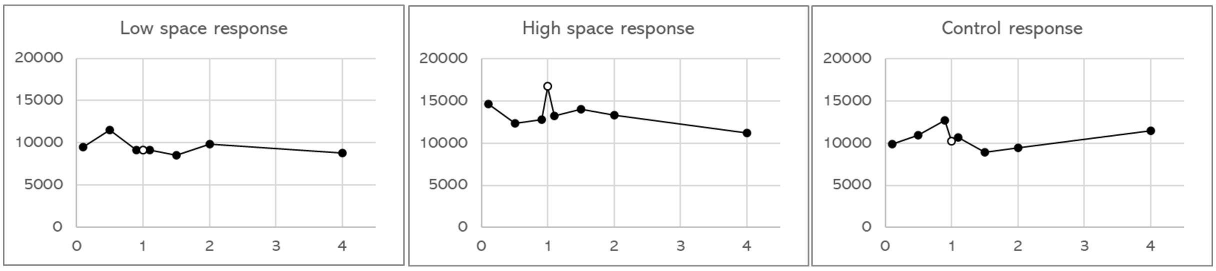


Fig. 90 Colony size after one year sensitivity to the proportion of drone eggs (DRONE_EGGS_PROPORTION) parameter changes for low space response, high space response and control response scenarios.

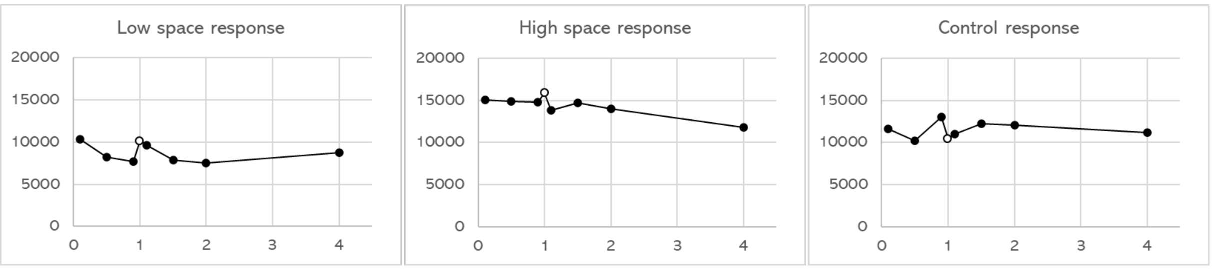


Fig. 91 Colony size after three years sensitivity to the proportion of drone eggs (DRONE_EGGS_PROPORTION) parameter changes for low space response, high space response and control response scenarios.

6.5.3.4. Maximum total distance a forager can fly on a single day (MAX_KM_PER_DAY)

Default value: 7,299 km

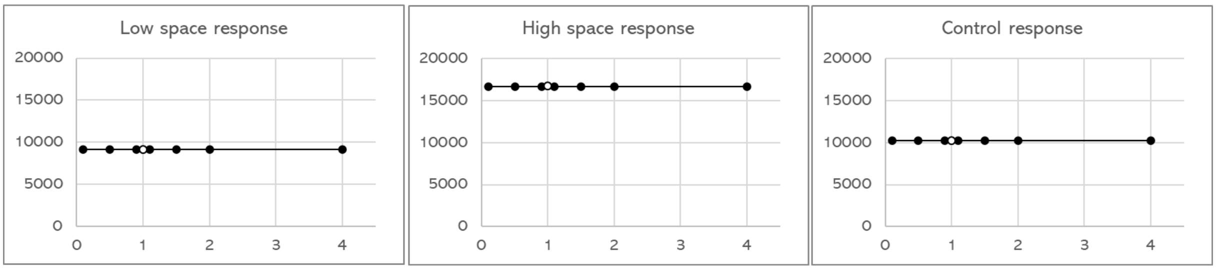


Fig. 92 Colony size after one year sensitivity to the maximum daily forager flying distance (MAX_km_PER_DAY) parameter changes for low space response, high space response and control response scenarios.

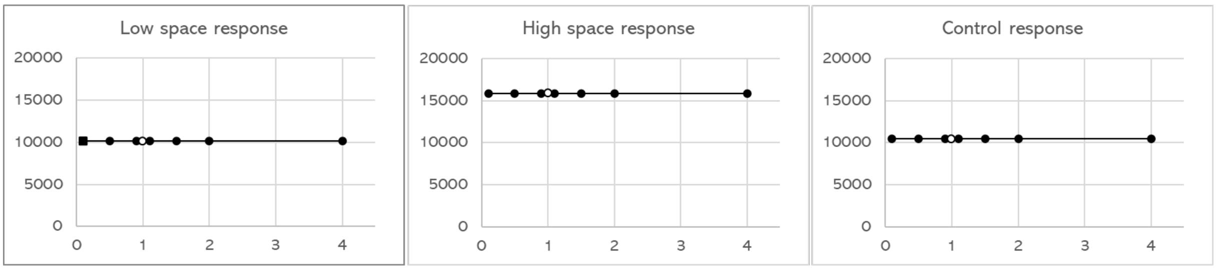


Fig. 93 Colony size after three years sensitivity to the maximum daily forager flying distance (MAX_km_PER_DAY) parameter changes for low space response, high space response and control response scenarios.

No effect in the analyzed model execution scenario (default model conditions) – most probably because of default food patch settings – shall be verified additionally in case of using a user-defined food source map. However, the parameter might be important in enclosed crop environments, especially the lower numbers. For that reason, this particular sensitivity analysis shall be repeated once such a habitat is defined and translated into the model.

6.5.3.1. The daily mortality rate of drone pupae (MORTALITY_DRONE_PUPAE)

Default value: 0.005 mortality/day

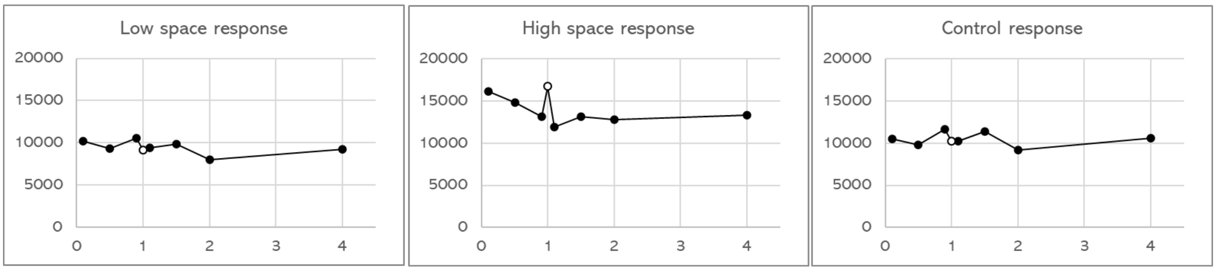


Fig. 94 Colony size after one year sensitivity to the daily drone pupae mortality rate (MORTALITY_DRONE_PUPAE) parameter changes for low space response, high space response and control response scenarios.

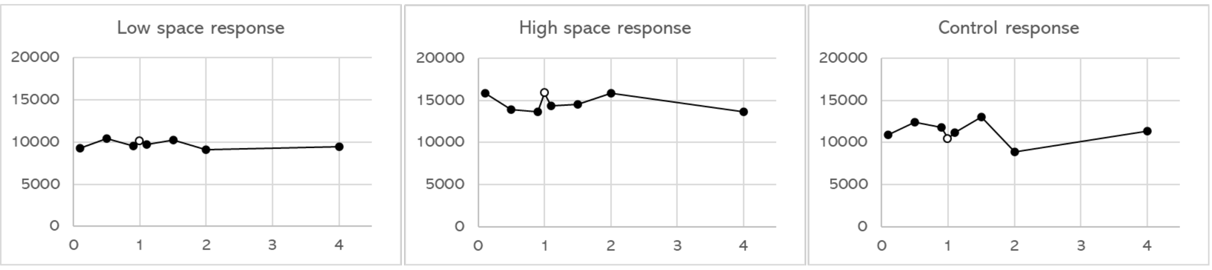


Fig. 95 Colony size after three years sensitivity to the daily drone pupae mortality rate (MORTALITY_DRONE_PUPAE) parameter changes for low space response, high space response and control response scenarios.

6.5.3.2. Daily mortality rate of drones (MORTALITY_DRONES)

Default value: 0.05 mortality/day

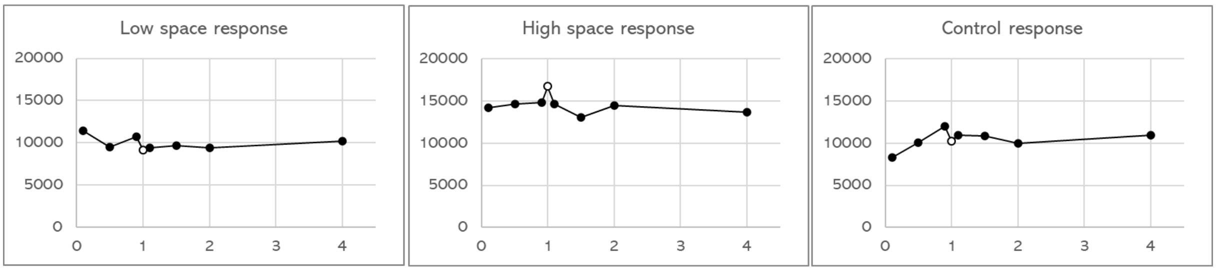


Fig. 96 Colony size after one year sensitivity to the daily drones' mortality rate (MORTALITY_DRONES) parameter changes for low space response, high space response and control response scenarios.

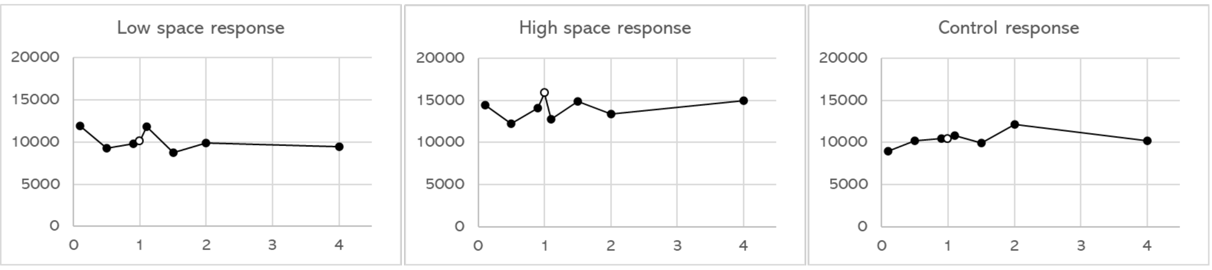


Fig. 97 Colony size after three years sensitivity to the daily drones' mortality rate (MORTALITY_DRONES) parameter changes for low space response, high space response and control response scenarios.

6.5.3.3. Daily mortality rate of worker larvae (MORTALITY_LARVAE)

Default value: 0.01 mortality/day

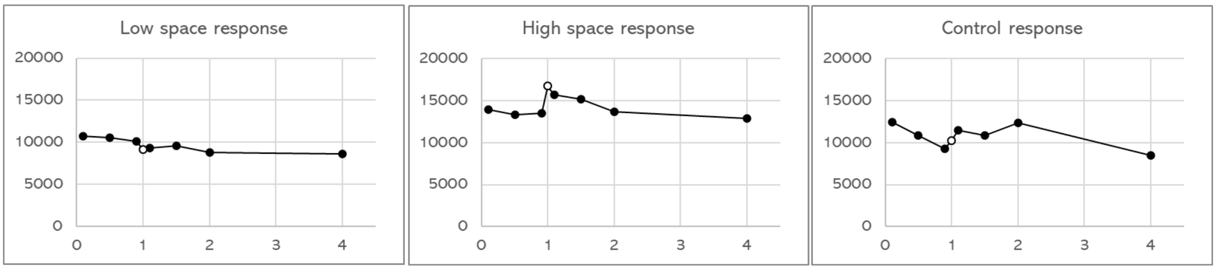


Fig. 98 Colony size after one year sensitivity to the daily worker larvae mortality rate (MORTALITY_LARVAE) parameter changes for low space response, high space response and control response scenarios.

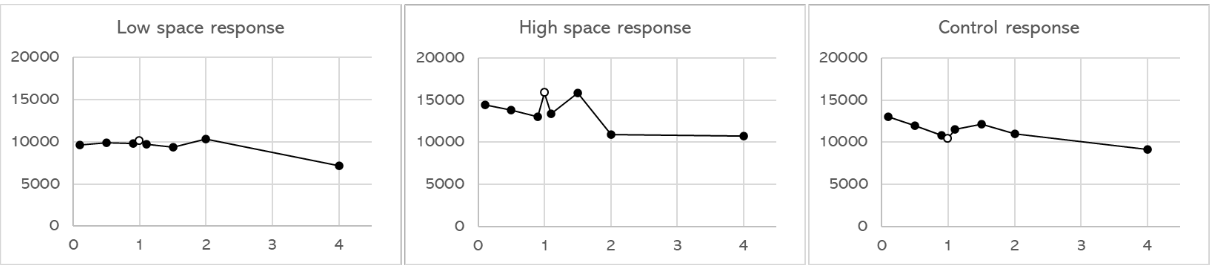


Fig. 99 Colony size after three years sensitivity to the daily worker larvae mortality rate (MORTALITY_LARVAE) parameter changes for low space response, high space response and control response scenarios.

6.5.3.4. Daily mortality rate of worker pupae (MORTALITY_PUPAE)

Default value: 0.001 mortality/day

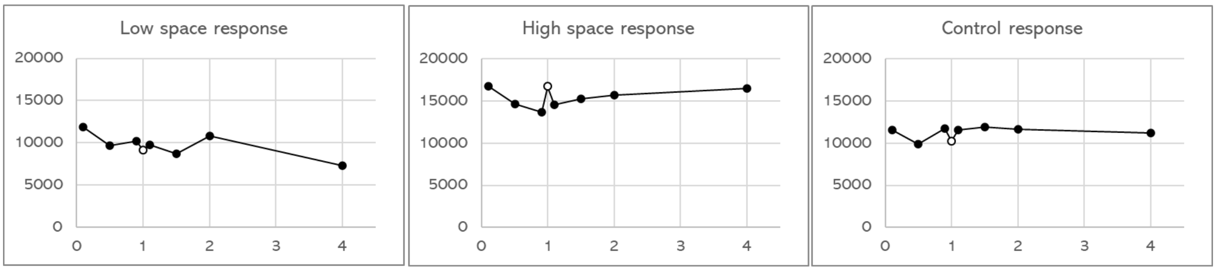


Fig. 100 Colony size after one year sensitivity to the daily worker pupae mortality rate (MORTALITY_PUPAE) parameter changes for low space response, high space response and control response scenarios.

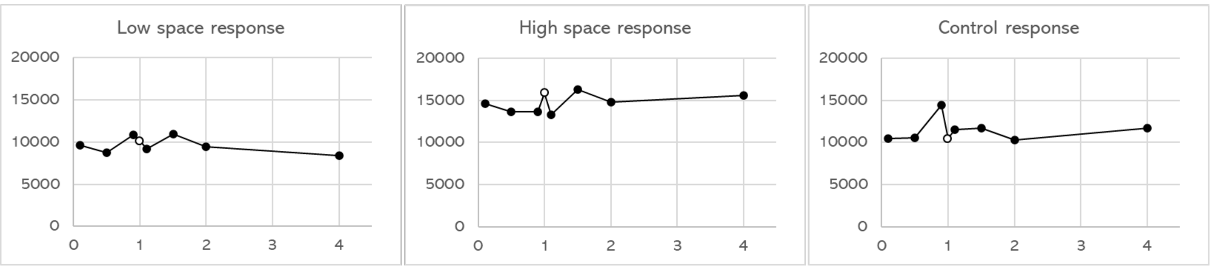


Fig. 101 Colony size after three years sensitivity to the daily worker pupae mortality (MORTALITY_PUPAE) parameter changes for low space response, high space response and control response scenarios.

6.5.3.5. The daily mortality rate of drone larvae (MORTALITY_DRONE_LARVAE)

Default value: 0.044 mortality/day

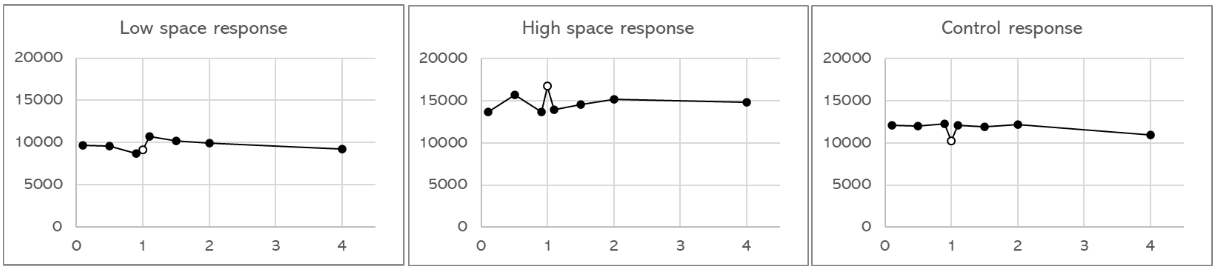


Fig. 102 Colony size after one year sensitivity to the daily drone larvae mortality rate (MORTALITY_DRONE_LARVAE) parameter changes for low space response, high space response and control response scenarios.

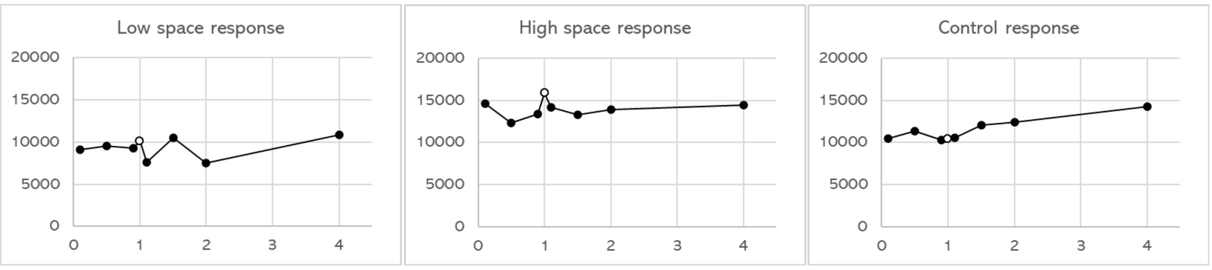


Fig. 103 Colony size after three years sensitivity to the daily drone larvae mortality rate (MORTALITY_DRONE_LARVAE) parameter changes for low space response, high space response and control response scenarios.

6.5.3.6. Time to unload nectar in the colony (TIME_UNLOADING)

Default value: 116 s

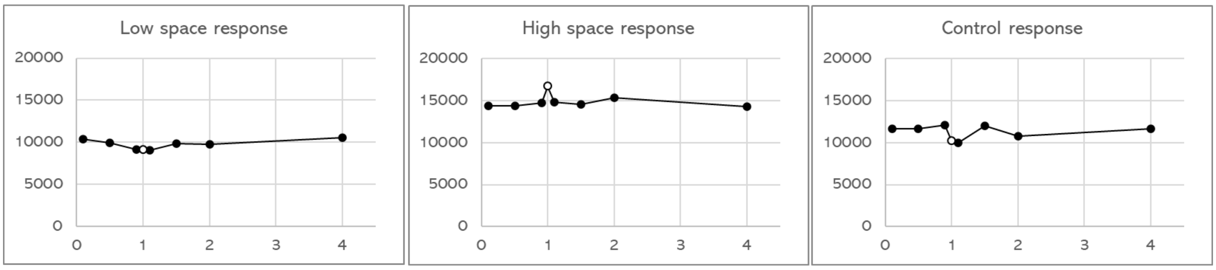


Fig. 104 Colony size after one year sensitivity to the nectar unloading time (TIME_UNLOADING) parameter changes for low space response, high space response and control response scenarios.

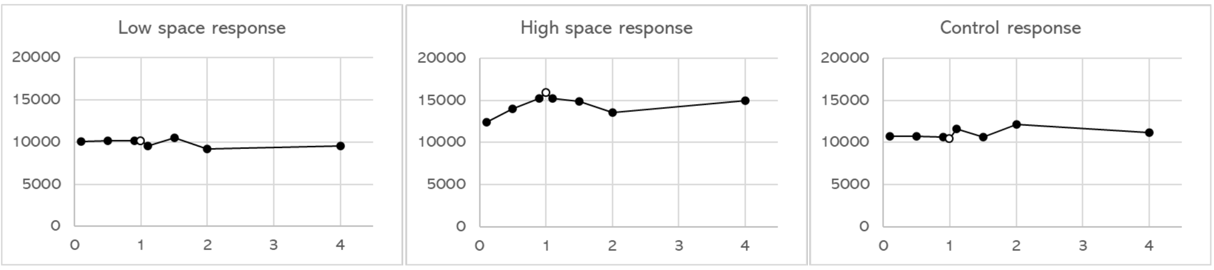


Fig. 105 Colony size after three years sensitivity to the nectar unloading time (TIME_UNLOADING) parameter changes for low space response, high space response and control response scenarios.

7. Discussion, conclusions and future directions

7.1. General remarks

The BEEHAVE is a relatively complex model that considers various processes with numerous interconnected modules and pictures the complexity of a honey bee colony's functioning. The complexity of its basic version enables the introduction of new modules based on limited data, with relatively good results, especially when each module considers the change of precisely one variable. Such an approach was used in the case of all the above-described modules, which gave results of reasonable accuracy despite the limited dataset they operated on.

The BEEHAVE_spacetravel model's basic version, operating on default configuration, provides stable predictions with the total colony size ratios for various scenarios that reflect the field observations well. Most of these differences come from the queen's egg-laying rates for various scenarios but operating with comparable dynamics. Also, the number of drones is visibly higher for the high space response scenario, similar to the real-life observations. Interestingly, the additional consideration of queen ageing affects the high space response to the greatest visible extent, additionally destabilizing the model's outcomes as more artefacts can be noticed. At the same time, the general egg-laying dynamics stay unaffected, with the maximum rate lowered in all cases by approximately 20%.

It was observed that the delay in the simulation start is beneficial to some extent – as shown in the analysis, when the simulation start was delayed for up to 60 days, the predicted maximum number of adults in the colony was greater than for the simulations starting on the first day of the year. However, starting the simulation on day 120 resulted in lowering the number again. Such results are being confirmed by the current beekeeping knowledge, indicating a strong relation between the introduction into the new season after overwintering, season-related food availability and competition of colonies [116].

As observed, the change in the hive type to the mini-plus model in some cases caused numerous colonies to store less food. Such behavior might be caused by the increased energetic requirements of colonies with numerous brood, due to the increased thermoregulation needs of the hive [117]. Space which is being freed from the food stores is then occupied by new eggs immediately, causing the observed disproportion to increase, especially in the case of the mini-plus hive of a very limited size. Additional analysis of the relation between hive type used and colony strength indicates the relation between honey harvesting and hive size – in the colonies from which honey was not harvested, the impact of the free space available in the hive, being strictly connected to the hive type used, was the strongest. In the case of colonies from which honey was harvested, this impact was visible to a limited extent due to the dominating impact of the harvesting itself.

The main purpose of the validation with the real-life field data was to test whether BEEHAVE_spacetravel could predict the initial effect sizes and long-term impact those initial effects had on colony dynamics for two space travel response scenarios with different modes of action, namely, maximizing or minimizing the queen's egg-laying rate.

As proven, the BEEHAVE_spacetravel, being equipped with the space response module, fairly well predicts the development of the colony in the year of giving the queen to the g-force. The validation against the experimental studies showed that it captures the initial effects on colony strength (total number of brood) and the subsequent colony dynamics well for both response scenarios. The model, considering all the available real-life data, such as hive type, feeding schedule, treatment timeline, initial age of the queen, and initial colony composition, predicted the relative magnitude of effects well at the colony level throughout the first year when started late in the season. Also, the condition of the colony and development rate after overwintering were predicted with satisfactory precision, comparable for default and real-life weather data, with slightly better performance for the default option. Additional consideration of the queen ageing caused the model to predict the number of brood to drop slightly later, improving both predictions based on default and real-life data. Furthermore, the queens' deaths during the first overwintering were predicted by most of the model starting data variations. The only model predicting the colony to survive winter but restart the activity again with a significant delay was the one based on the control sample data with real-life weather and no ageing considered. Such highly specific conditions required by the model for the survival prediction support a hypothesis that the most probable cause of such a model's behavior was very specific weather conditions during the field study. Another aspect supporting the above is the observation that consideration of local weather conditions in several cases resulted in significantly more accurate predictions than default weather settings. This is reflected in the real-life colonies' behavior, which is strongly related to the local weather conditions [118], [119]. Such a conclusion might be additionally supported by the slight differences in response to unfavorable weather conditions of various honey bee species – *A. mellifera* usually undertakes flight activity in lower temperatures than *A. dorsata*, for which minimum air temperature must exceed 18°C [120].

The model's predictions of good accuracy were also observed for the pupae number and number's trend, while predictions for eggs were of the worst accuracy. Similar to the weather scenario, such a model's response also finds a good explanation for colony behavior. Eggs are the brood type related to the smallest "energetic cost", covered by the cost of laying egg by the queen and the cost related to the temperature management by nursing bees. In the case of brood, especially pupae, in addition to the aforementioned energy costs, there is also the additional expenditure associated with the need to feed it, and thus the previous necessity of foraging. For that reason, in case of the need to limit the colony's size, workers start by limiting the number of eggs, e.g. by eating them [121]. Such a decision may be caused by various environmental factors, such as limited nectar or pollen source, unfavorable weather conditions, swarming, etc. [121]. This observation might indicate the possible need to expand the model with feedback loops, enabling the consideration of in-hive food dynamics in the future, as it might significantly impact the accuracy of the number of eggs predicted.

Although the relative effect sizes for colony strength were captured well by added space response modules, the absolute numbers for specific bee development stages were less accurate. The lack of accuracy was mainly caused by the scarce field data, presenting the development of one colony rather than the general trend resulting from several colonies' dynamics. Thus, BEEHAVE_spacetravel underpredicted the total number of cells with eggs and pupae for both space response scenarios, and this was reflected in the hive-type part of the simulation, where BEEHAVE_spacetravel was strongly limited by the limited space available in the beehive.

The threshold colony size necessary for winter survival shall additionally be verified in future experimental studies on the topic, as in many simulations performed with real-life weather data, colony collapse was predicted. Such a prediction is not in line with the observations made during the field study [122]. Apart from the previously mentioned temperature resilience of the workers, one of the factors which might have caused such a model's behavior, could be the critical colony size being set to 4,000 workers.

The observations described above were even more visible when the field assessment data was used as read-in files for the model. In the case of predictions made for the low space response colony, combining the read-in file with real-life weather data caused the simulation to switch from winter collapse to winter survival, being in line with field observations. While the analogous procedure had no effect on the high space response colony, it caused the exact opposite effect in the control sample. The possible explanation may be the fact that input data for low space colony came from exactly one colony while control sample data was based on a mean value data of two control sample colonies. When analyzed separately, those control colonies were both predicted to winter collapse despite the used scenario. The observation still requires more field data to become available to verify which tendency is shown by the model more frequently and to mitigate this trend in future versions of the model's code.

Based on the described observations, field colony assessments should be performed whenever possible and within reach of the time/budget/human resources. Such input data is invaluable for the model and can improve its performance in terms of the accuracy of the winter survival prediction.

Apart from being used for the colony development prediction, the model can indicate the further space conditions impact on honey bee colony development research directions – as shown in sensitivity analysis. The model is more sensitive to changes in several specific parameters. For example, due to the model's significant sensitivity to changes in the time of nectar and pollen gathering, these parameters shall be particularly well studied under altered gravity conditions, as flight and foraging activities of bees might be affected by the difference in gravity. Similarly, the model's sensitivity for the volume of the forager's honey stomach and maximum worker lifespan can be an indication to verify the parameter for workers born in extraterrestrial conditions and/or developed from eggs laid by the queen, which was given to the space travel/hypergravity conditions, especially when considering possible lowering of those values. Additionally, the model's sensitivity highlights the possible criticality of proper design and composition of extraterrestrial greenhouse and crop by its sensitivity to the amount of pollen collected by foragers during a single trip. This particular parameter, in the case of an artificial, enclosed food production system that is entirely reliant on human factors, impacts the overall colony strength, particularly for values lower than the default.

As previously discussed, and additionally shown in Chapter 6.4.6, in its current form, the model is unsuitable for predicting the colony's introduction into the new season after an overwintering period. Such predictions are of very low accuracy, with the best results noted for the low space response scenario. However, even in that scenario, the model's accuracy was limited to its ability to predict correctly only the trend of the changes but not its magnitude. Moreover, in the case of including real-life weather data in the analysis, the model failed to predict colony overwintering survival, proving its unsuitability for this specific purpose.

The model's accuracy improves for the low space response scenario in case of additional consideration of the read-in files containing the brood numbers from the field observations. However, such improvement has a limited possibility of being used in real-life situations, as there would exist a need for the beekeeper to not only know the number of brood on the first day of the simulation but also to conduct continuous colony observations throughout the beekeeping season to collect such data. To improve the model's predictions significantly, such observations would require precision and, therefore, would lead to a significant increase in required labor, unreasonable for a non-research apiary.

One of the BEEHAVE submodels enables the definition of custom food patches. This feature enables to picture the real availability of food in the environment with very high accuracy, particularly in the case of enclosed artificial ecosystems. This, in turn, opens the possibility of verifying if the available food sources are sufficient for the bee colony's needs and whether their insufficient quantities will lead to its premature collapse.

In summary, hypothesis H1 was partially confirmed, entirely proving the hypothesis H2. Even though the model is based on very limited data, it was proven it could correctly predict the colony development dynamics, and it was shown that its accuracy is better when more real-life data is included in the analysis. However, the repeatability of the model's correct predictions still needs to be verified on a larger dataset. Nevertheless, the BEEHAVE model has great potential, as shown in the thesis and proved by other non-space-related works. To use it fully in the context given in this dissertation, some priority areas require further investigation and inclusion, such as the impact of lowered gravity, e.g. lunar or Martian, on worker bee morphology and foraging performance or establishing the precise egg laying rate of the queen subjected to hypergravity.

The results presented in this dissertation, along with the current simulation and space biology studies and trends, highlight the possible directions for future work. There still exist a number of gaps, which, once filled, will improve modeling precision and, as a consequence, will enable the enhancement of the understanding of extraterrestrial crop pollination possibilities. For now, there are no specific requirements for the rocket transportation of pollinators. More experiments similar to the one described in Chapter 4 will not only provide more input data for similar simulations in the future but might also be used in the future to create requirements on the maximum g-load factor acceptable during the space transportation of bees. To ensure sufficient reliability of the data for the regulatory context, the research should broaden the scope of the researched pollinators with other, already widely used in crop pollination species such as red mason bees (*Osmia rufa*) or bumble bees (*Bombus*)

7.2. Model limitations

The first and foremost limitation of the model is that it is based on very scarce data. The most important flaws of the data, other than their quantity, include not covering a full beekeeping season, the start of colony development observations in August after the maximum reproductive activity of the queen, or the lack of a complete picture of the colony's entry into the new season after overwintering. Despite the reasons for such a study design, a similar approach should be avoided in future follow-up studies to ensure greater data reliability and, as a consequence, improve the model's credibility and accuracy. For that reason, in the current shape model should not be used for any final design decisions or legal actions and should be treated only as a guidance and suggestion in determining areas requiring more in-depth analysis.

Moreover, existing data focuses only on the very initial part of space travel, the rocket launch. It omits entirely the impact of subsequent phases of travel and the associated possible unfavorable conditions, such as the presence of microgravity or lowered gravity conditions or increased exposure to cosmic radiation. Study continuation and expansion is required to fill these gaps.

In the basic version, the egg-laying procedure is based on a bell-shaped curve without random variation added. In such a form, the egg-laying rate is stable as long as empty cells are available. This specific number can be modified, but changing the number throughout the season is impossible. The number of bees available for brood care can additionally limit the egg-laying rate. However, as shown by sensitivity analysis, any slight change in the number of cells under the care of a single nurse bee causes significant changes in the general model's output. The queen's ageing and introduced space travel effects were the biggest impact on the parameter.

As mentioned above, no changes in the hive size during the season are possible in the current form of the model, being at odds with established beekeeping practices, which recommend adjusting hive size to the needs of the colony. Adequate intervention can even stop the development of a swarming mood in the colony or induce the queen to become more or less active.

Another important limitation of the model is the inability to simulate the number of crops pollinated by the specific colony expressed by the crop yields acquired. Such functionality would be particularly useful in the context of highly demanding conditions, such as extraterrestrial, and would explicitly determine the profit of non-bee-products from the development of a particular honey bee colony. However, overcoming this limitation will require providing data to the BEESCOUT part of the model on the available food patches, most probably by expanding the capabilities of the BEESCOUT itself in terms of the maximum number of patches considered. Moreover, a highly specific and extensive field study will be required to establish the real relation between the number, duration and ratio of bees' visits to flowers with the later quality of yield, expressed by fruit's weight, measurements, palatability or combination of all three, and quantity of the yields. Efforts to identify such relationships are being undertaken, and a meta-analysis performed by Rollin and Garibaldi summarizes the current knowledge and gaps in the topic [123].

Finally, the limitation of the presented research is the lack of possibility to simulate greenhouse conditions. This constraint can be partially compensated by using very precise and specific weather and food patch data. However, equipping the model with the dedicated “greenhouse module” with presumed internal conditions and food source composition would be a great simplification for future model users and could enhance the model promotion among new groups of users.

8. Summary of the most important accomplishments of the dissertation

Despite its limitations, presented research for the first time addressed the topic of the impact of space travel on condition of the pollinators. As shown by the literature review, there is virtually no information on how rocket launches influence pollinators, especially, honey bees in the long-term. To fill the tremendous gap in our knowledge, I designed the first of such experiments to gather the data necessary for the creation of the model, enabling the prediction of the rocket transportation-related effects on honey bee colony development. It enabled to create the first database focused on the impact of space travel-related hypergravity on *A. mellifera* fertility, enriching humans' knowledge of space travel's impact on pollinators physiology. Results of the experiment are publicly available, along with the article describing the research methodology [109]. The device used during the experiment can be used in future studies, aiming to enrich the mentioned database, ensuring the experiments' conditions are the same across different studies [33], [49]. Usage of the developed device in future studies will enable to limit the number of conditions varying across them.

The model I created is the first one comprehensively taking into account various factors affecting honey bee colonies in space context. I parametrized, analyzed, and compared it with the available data in terms of its accuracy and suitability for usage to predict the effects of giving honey bee queen to the stress associated with the rocket flight. I confirmed the partial compliance and established the most important areas for further development and improvement, with indication of the suggested methodology.

I performed a critical analysis of the model outputs in comparison with the gathered real-life field data, with the consideration of the current beekeeping and biological knowledge, considering the BEEHAVE model's assumptions and rules of work and in regard of the possible future extraterrestrial settlement needs and directions. The combination of the topics considered in the analysis resulted in a unique perspective, unachievable in any other expertise configuration.

My research has uncovered many important aspects of modeling honey bee colony in the context of the effects of space travel on its development. I found that the model is more stable for scenarios considering limiting of the queen bee in the number of eggs laid and tends to be more unstable and have more artifacts for the high space response scenario. Additionally, I proved that in the current form model is not suitable for predicting introduction of the colony into new season after overwintering period.

I believe all the above-mentioned accomplishments will enrich current space biology and biocybernetic modeling knowledge, leading to further development of the fields. I am convinced that the results presented in this dissertation will significantly affect our planning in the context of extraterrestrial settlement.

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