Kaye Bonto
ZOOL 567 F2021

Literature Review 3: Annotated Bibliographies

Legend
Nutritional (or food) stress
Predation
Immune challenge (including pathogens)
Heat stress

This compilation of annotated bibliographies is organized with the secondary review article as the first entry. Primary articles are color-coded depending on the type of environmental stress explored in the study and subsequently arranged chronologically based on publication year.

Klein, S., Cabirol, A., Devaud, J.-M., Barron, A. B., & Lihoreau, M. (2017). Why bees are so vulnerable to environmental stressors. ***Trends in Ecology & Evolution*****,** ***32*****(4), 268–278. https://doi.org/10.1016/j.tree.2016.12.009.**

Article Summary |

This article is a summary of studies that consider why bee populations are declining because of their sensitivity to environmental stressors. It discusses the actions of stressors on the nervous system and its effect on cognition as a possible cause colony decline. These stressors are predominately anthropogenic, such as the increased prevalence of pathogens and parasites, pesticide use, and environmental degradation.

This article argues that foraging places high demands on cognitive function. Honey bees need to learn to forage efficiently by finding high-quality patches, efficiently handle flowers, and navigate back to the nest to communicate resource location to their nestmates. Specialized brain regions involved in these complex tasks such as the MBs (mushroom bodies) and CX (central complex) are known to be affected by pesticides containing neonicotinoid and fipronil as well as heavy metals such as manganese. Chronic exposure can drastically affect bee cognition by either impairing the development of MB or affecting MB plasticity that can lead to the disruption of memory, navigation, and learning, which are essential for successful foraging. Intensive farming and monocultures can decrease the diversity of food sources for bees, and can in turn disrupt associative learning, such as the combination of visual and odor cues when foraging.

The impairment of cognition and foraging performance due to stress results in an overall decrease in fitness because it reduces bees' capacity to rear successful workers to maintain its population. Foraging prematurely is a common behavioural response to stress, resulting in inefficient foragers that can result in a population collapse if exposed to prolonged stress. Given this conclusion, this review highlights the advantages of embodying an integrated research approach that combines the fields of neuroscience, ecology, and evolution as it better reflects the cascade of effects of stress on bee colonies.

Article Contribution |

This review article provides a neurological, ecological, and evolutionary overview as to why bees can be highly sensitive to environmental stress. It highlights the concern about human-induced stressors that increases with industrialization over time that can lead to a pollination crisis if not mitigated. Combining research in neuroscience, in how stress affects cognition, ecology and evolutionary biology, in how stress can disrupt colony dynamics, allows for an inter-disciplinary review approach to help identify an effective intervention to alleviate stress on bees. It also leads into new questions such as the extent to which stress can affect honey bee communication.

Mayack, C., & Naug, D. (2015). Starving honeybees lose self-control. *Biology Letters*, *11*(1), 20140820. https://doi.org/10.1098/rsbl.2014.0820.

Article Summary |

Previous studies have put forth two paradoxical hypothesis behind impulse control in honey bees, where one, links high metabolic rates to increased starvation risk and poor self control and two, that argues a social hypothesis that self-control is exhibited to maintain harmony in the colony. Thus, this study attempts to investigate whether impulse control is driven by individual energy states, the colony state, or influenced by both, by testing the effect of starvation on impulsivity when removed from its social environment.

A proboscis extension reflex (PER) assay was conducted to observe the effect of starvation on impulse control on individual honey bees. To test impulsivity, researchers first conditioned bees to associate odours to rewards of different qualities. Bees were starved for either 6, 18, and 24 hours and after each time stamp, bees were subjected to the PER assay. Choice was noted for bees that extend their proboscis towards the food source. Biogenic amine levels were also quantified by using high-performance liquid chromatography (HPLC) on bee brains to determine the amount of biogenic amines at specific starvation stages.

Their findings were more consistent with the first hypothesis where honey bees were found to exhibit self-control in high energetic states and were more impulsive when starved. Moreover, they found that it depended primarily on individual bees' energetic states, which is significant especially in a eusocial insect like the honey bee, as it can potentially instigate intracolonial conflict and disrupt the harmony within the group. They also found that other brain amines were not significantly different with starvation, but dopamine was, which denotes the significance of this study as the first to link the increase in dopamine levels to impulsivity. A future study suggested could be to investigate the probable plasticity of impulsivity, wherein other energetic stressors may also cause impulsive behaviour.

Article Contribution |

This article adds to current literature on the ability of stressors to cause behavioural changes in honey bees. More specifically, it investigates the effect of starvation on cognition and impulse control, which are important for honey bees to make advantageous foraging decisions. It supports previous findings that impulsivity is driven mostly by individual energetic state; however, this study was unable to refute the social hypothesis, that impulse control is favoured for group fitness. They conclude that both hypothesis may be true to some degree, and future evolutionary studies may be conducted to determine how impulse control in social bees evolved.

Scofield, H. N., & Mattila, H. R. (2015). Honey bee workers that are pollen stressed as larvae become poor foragers and waggle dancers as adults. *PLOS ONE*, *10*(4), e0121731. https://doi.org/10.1371/journal.pone.0121731.

Article Summary |

The negative effects of juvenile nutritional stress on adult behaviour, that is widely researched among vertebrates, is poorly understood in invertebrates. This article investigates the impacts of larval pollen stress on adult foraging and recruitment behaviour in honey bees. Scofield and Mattila (2015) explored this by splitting source colonies into three subunits per colony, with one pollen limited or nutritionally stressed subunit and two controls that were abundantly supplied with pollen. This was conducted in three trials over two years, in a similar social environment. The pollen limited subunit contained 1-2 frames of $<50\ cm^2$ of pollen-filled comb and 3-4 with only honey while the second subunit had a similar set up except it had one 90% pollen-filled frame. Both were confined and prevented from collecting pollen from the environment. The third subunit had the same amount of pollen as the previous subunit but was unconfined and can forage for pollen in the environment. Weight, longevity, foraging activity, and waggle-dance behaviour were observed in each trial subunit.

They found that foraging and recruitment behaviours of adult workers reared in a nutritionally deficient environment in their larval stage were compromised. Honey bees under food stress were smaller and had a shorter life-span when compared to colonies abundantly provisioned with pollen. Early onset foraging was observed, wherein workers foraged infrequently and were more likely to die after. Pollen stressed bees were less likely to waggle dance and if they did, their dances were observed to be less precise. These results were significant since it confirms that environmental stressors that limit bees' access to nutrients can mirror the effect of juvenile undernourishment in this study. This suggests the possibility of environmental stressors acting synergistically that may be of interest for researchers aiming to provide interventions to prevent further population decline.

Article Contribution |

This article adds to current literature on the negative effects of juvenile undernourishment on adult behaviour among invertebrates, specifically on honey bees. This study also provides a more realistic assessment of the effect of food stress by observing it in its natural social environment, compared to previous studies that have combined artificial rearing practices. This article was chosen since it explores both the physiological and behavioural changes in individual honey bees and colonies. It also proposes the possibility of a synergistic interaction between poor nutrition and other environmental stressors that can ultimately disrupt colony function.

Perry, C. J., Søvik, E., Myerscough, M. R., & Barron, A. B. (2015). Rapid behavioral maturation accelerates failure of stressed honey bee colonies. ***Proceedings of the National Academy of Sciences*****,** ***112*****(11), 3427–3432. https://doi.org/10.1073/pnas.1422089112.**

Article Summary |

Extensive studies have been conducted on effect of individual stressors such as food scarcity, pests, pathogens, and agrochemicals, however, it remains unclear why behavioural adaptations such as precocious foraging can result in a drastic decrease in honey bee population. This article investigates why colonies undergo dramatic population collapse in response to stress, proposed a method of assessing colony health, and also suggested possible ways to mitigate colony collapse.

Three single cohort colonies (SCC) with 1 day old adult honey bees were created as well as three normal-worker demography colonies, wherein SCC contains a younger foraging force. Radio frequency tags (RFID) were used to monitor foraging performance and how it varies with age within the two colony types. A honey bee population dynamics model was created to model the potential consequences of precocious foraging within colonies with the availability of food and survival per foraging as a function of age. One trend that is consistent with previous literature is that honey bees adapt to environmental stressors by maturing faster and foraging early. This may buffer impacts of short-term stress, however, in chronic stress an inefficient and younger foraging force was found to be unable to sustain the food requirements of the colony, potentially leading to a sudden population decline. This study is significant because it is the first to hypothesize and demonstrate with a model that bee foraging performance varies with age. Moreover, it is the first to display the complex social dynamics of colony collapse and suggest a more accurate assessment of colony health, through food stores and brood production. A future direction that goes beyond protein (pollen) deficiency as a source of nutritional stress but also includes carbohydrate (honey) is suggested which might expand current understanding of its effect on population dynamics.

Article contribution |

This article enhances current literature by modeling situations wherein honeybee colonies might be at risk of rapid population decline by observing the impact of stress on foraging behaviour and their adaptations in response to chronic stress. Previous studies have established that honey bees adapt to stress by foraging early, however, this study expands on this by explaining that colony collapse occurs from social responses of honey bees to stress. By combining an experimental and model-driven approach, this study has synthesized a novel and systematic way assessing colony health and informs future research on possible stabilizing interventions to prevent colony collapse.

Wang, Y., Kaftanoglu, O., Brent, C. S., Page, R. E., Jr, & Amdam, G. V. (2016). Starvation stress during larval development facilitates an adaptive response in adult worker honey bees (Apis mellifera L.). *Journal of Experimental Biology*, *219*(7), 949–959. https://doi.org/10.1242/jeb.130435.

Article Summary |

Previous research conducted on model organisms have suggested that exposure to juvenile stress can influence an offspring's genetic development, by allowing them to develop physiological and behavioural mechanisms to combat stress in adulthood. This study aimed to experimentally confirm if an anticipatory mechanism does develop at a larval stage when starved that allows honey bees to adapt to food scarcity in adulthood.

Three-wild type colonies were divided into two treatment groups: (1) control and (2) starved. To induce starvation, honey bees were separated using exclusion cages for 10 h to prevent co-mingling and nurse bees from feeding the starving larvae. Body mass of <24 h and 7 day old adults and their ovariole numbers were recorded to validate the effect of starvation on growth and reproductive ability. Hemolymph, fat body, juvenile hormone (JH) levels, and gustatory perception of sugar were all collected at both adult stages. Adult body mass was found to be significantly different for adults <24 h old and were observed to have a significant reduction in ovariole number. These results are consistent with previous studies in insects that showed trade-off in reproduction to allocate for other strategies to cope with stress. An increase in glycogen in 7 day old bees is significant since glycogen stores can supply energy demands under stress. An increase of glycogen in adult honey bees could be predictive of their ability to sustain themselves under food stress. Early food deprivation resulted in a significant increase in JH titer when bees were starved and stayed elevated throughout adulthood. Nutritional stress was also found to permanently affect gustatory perception, which significantly alters their ability to discern high reward food patches when foraging. A future direction provided is to investigate possible changes at a colony level, especially in terms of division of labour in honey bees.

Article Contribution |

This study is significant in demonstrating the complex, physiological, behavioural, and hormonal changes that occur when larval honey bees are exposed to nutritional stress. It also supports previous studies that denotes trade-offs in energy expenditure in order to adequately adapt to stress (Bordier et al., 2018), specifically between reproduction and body maintenance. This research advanced the field by investigating the long-term effects of environmental stressors in studying honey bees from its larval to adult stages. Since this study focuses more on the effect on individuals, a future study exploring the effect of long-term stress at the colony level is suggested.

Bateson, M., Desire, S., Gartside, S. E., & Wright, G. A. (2011). Agitated honeybees exhibit pessimistic cognitive biases. *Current Biology*, *21*(12), 1070–1073. https://doi.org/10.1016/j.cub.2011.05.017.

Article Summary |

Pessimistic cognitive bias pertains to the bias in information processing resulting from negative states such as anxiety, wherein animals have increased expectation of punishment or misconstrue harmless stimuli to potential threats. Previous studies on mammals and birds have showed correlation between juvenile stress, poor stress response in adulthood, and pessimistic cognitive biases in decision making when exposed to stressful situations. Honey bees' ability to categorize sensory stimuli and correlate them with quality of reward or anticipate consequences through associative learning, as established by previous studies, makes it a prime model invertebrate to study the effects of anxiety-like states on their display of cognitive biases.

The measurement of cognitive biases in honey bees were modeled after previous vertebrate studies wherein prior to formal testing, honey bees underwent operant conditioning to learn to identify stimulus that is associated to reward (CS+) and punishment (CS-). After this training, half of the subjects were physically agitated through shaking to simulate stress from a predator attack. They observed that stressed bees under unreinforced test trials extended their proboscis on CS- flowers, which denotes that their association of food rewards and odor cues were significantly altered after exposure to stress. Moreover, hormone levels were measured, and they found that octopamine, serotonin, and dopamine levels were significantly reduced which could directly explain cognitive bias since these hormones have been found to be involved in learning. These results are significant because it demonstrated that stress from a simulated predatory attack can have a negative effect on cognition and subsequently on foraging behaviour. This confirms the hypothesis posited by previous studies that stressors can act synergistically. Since studies on cognitive biases in invertebrates are still currently underdeveloped, physiological mechanisms that drive this behavioural change could be a potential future direction for this study.

Article Contribution |

This article supports previous studies about the possible synergistic interactions between environmental stressors leading to adaptive behavioural responses. This study is significant because it is the first to demonstrate relationship between stress in bees and pessimistic cognitive biases when foraging. Thus, their findings propose a method of classifying stress in honey bees by observing the presence of pessimistic cognitive biases and correlating that with states of anxiety. A question that arises is if whether this only occurs at an individual level, or if it also affects eusociality like the division of labour in honey bees.

Goblirsch M, Huang Z.Y., & Spivak, M . (2013). Physiological and behavioral changes in honey bees (Apis mellifera) induced by Nosema ceranae infection. *PLOS ONE, 8*(3), e58165. https://doi.org/10.1371/journal.pone.0058165.

Article Summary |

The division of labour among honey bees have been known to be driven by the organization of workers into castes based on age. Nurse bees are comprised of workers that are less than three weeks of age and is responsible for feeding and maintaining hives. The transition into foraging is typically at the end of the honey bee life cycle, typically surviving for another two to three weeks. As previous studies have also explored, environmental stressors such as predation, malnourishment, and immune challenges can disrupt this precarious caste division. This study looked at a specific pathogenic stressor, *Nosema ceranae*, which can cause severe infection in hives and threaten colony survival. Its objective was to investigate how infection by *N. ceranae* can disrupt physiological factors that is allows for division of labour to occur within a colony.

Honey bees were inoculated with *N. ceranae* spores through oral administration of a mixture of spore and sucrose solution. Mean percent survival was determined by recording the number of dead bees that were removed daily in each cage. To investigate the alteration of foraging behaviour post-inoculation, paint-marked bees between ages 7 and 21 days were observed daily for 30-mins if they contained pollen loads, which is indicative of precocious foraging. Vg (vitellogenin) and JH (juvenile hormone) levels were quantified via qRT-PCR and radioimmunoassay, respectively. They found that percent survival of spore-infected bees decreased significantly compared to controls. *N. ceranae* infection did result in premature foraging, where significantly more bees foraged at precocious ages of 7 to 21 were observed in the infected group. JH and Vg levels showed an inverse relationship which is indicative of age-specific caste system in honey bees, where high Vg is correlated to reproductive investment in young nurse bees and high JH to the transition to foraging.

Article contribution |

This article proposed a proximal influence on the division of labour and caste system in honey bees. It is consistent with previous studies that showed that stress can alter foraging behaviour, wherein young bees mature early and forages precociously. This is a significant concern especially with stressors acting on colonies synergistically, as proposed by multiple studies. Poor foragers can result in a colony decline, especially with precocious foragers having a much shorter lifespan as indicated by this study. Since *N. ceranae* significantly affected infected caged bees, this article is significant when considering the risk of pathogenic stress in welfare facilities.

Bordier, C., Klein, S., Le Conte, Y., Barron, A. B., & Alaux, C. (2018). Stress decreases pollen foraging performance in honeybees. *Journal of Experimental Biology*, (4), jeb.171470. https://doi.org/10.1242/jeb.171470.

Article Summary |

Previous studies on the foraging behaviour of honey bees found it to be energetically taxing as it requires high cognitive and metabolic capacities. Therefore, trade-offs in energy expenditure can occur because of exposure to stressors, specifically through behavioural adaptions such as by decreasing foraging performance or altering foraging decisions. This article aims to expand on this hypothesis by investigating the effects of immune challenges on foraging behaviour and on hormones that regulate it.

Two sets of experiments were conducted to observe the impact of immune challenge (or pathogenic stress): (1) on foraging performance and (2) impact on brain biogenic amine levels. These experiments were performed over four months wherein focal bees' immune system were activated by piercing their abdomens. For experiment 1, bees were tracked using RFID tags to record their foraging trips. Brain dissections were conducted on successful foragers to measure biogenic amines such as octopamine, dopamine, and tyramine. They found that the survivorship of immune challenged bees did not differ significantly from control, however, the foraging performance of stressed bees were significantly altered in that they foraged longer and less often.

Dopamine (DA) and tyramine (5-HT) levels were not significantly different while octopamine significantly decreased in stress bees, which could explain slow foraging, since previous studies have associated OA with flight. These results are significant in considering the detrimental effects of prolonged nutritional deficits at the colony level. Moreover, this study proposes that stressed bees could potentially offset the energetic cost of pathogenic stress by preferentially choosing a carbohydrate-rich nectar over protein-pollen which could negatively affect brood care and larval development. Experimental studies involving the manipulation of OA, DA, and 5-HT may be of interest for future research to determine the proximal influence of these amines on foraging decisions and behaviour.

Article Contribution |

With the increasing prevalence of pathogens and viruses, this study is significant in raising concern about the threat of immune challenges on the health of honey bee colonies. This article supports previous studies that have hypothesized of the synergistic effect of multiple stressors on colony health, wherein immune challenges can cause a decrease in foraging performance leading to malnutrition and negatively affect brood care and survivorship within colonies. Moreover, the energy expenditure hypothesis advances knowledge in the field since it allows for observational studies using ethograms to potentially categorize behavioural indicators of stress.

Johnson, B. R. (2002). Reallocation of labor in honeybee colonies during heat stress: The relative roles of task switching and the activation of reserve labor. Behavioral Ecology and Sociobiology, 51(2), 188–196. https://doi.org/10.1007/s00265-001-0419-1.

Article Summary |

Previous studies have observed that reallocation of labour occurs in social species, when exposed to environmental stressors. There remains a gap in understanding about how honey bee colonies can organize workers to do specific tasks especially under stress. This article focuses on heat stress, which is one critical stressor that can cause abnormalities in larval development. The main objective of this study is to confirm how labour is reallocated at both the colony and individual levels in response to heat stress.

This study was conducted over two months wherein four colonies of bees were marked and allowed to acclimate to their social environment. Observations were made at the onset of foraging noting each behaviour (eg. tongue lashing, self-grooming etc.) at a specific timed interval. Space heaters were used to manipulate the temperature and induce heat stress, which is defined to be over 36ºC. At the group level, specialist tasks like wax working decreased especially in the afternoon while standing (or looking for work) increased. Behaviour adaptations to heat that promotes cooling also increased such as fanning and tongue lashing. Auxiliary tasks such as self-grooming decreased significantly which confirms the hypothesis of reallocating labour to adapt to heat. The observation that at least 50% of the honey bees appeared to be disoriented looking for work, task-switching, or quitting is significant since it supports the hypothesis that stress can upset the division of labour and the caste organization within bee colonies. The frequency and mechanism of task-switching remains unclear following the study and is proposed to be a good future direction for research. Johnson (2002) hypothesizes that frequent quitting may be due to a hierarchy of stimuli that honey bees' experiences and thus task switching can be categorized as an organizational mechanism similar to division of labour seen in social species.

Article Contribution |

This article extends the discussion on the effect of stress on the division of labour in honey bees through reallocation of labour as a behavioural adaptation. It is consistent with previous studies, especially with the energy expenditure hypothesis regarding effect of stress on the division of labour and caste system of honey bees. Furthermore, it explores heat stress, which is unique compared to the other environmental stressors explored in this review. This article's reliance on ethograms makes the application of this study accessible and easy to replicate, especially for welfare facilities looking to define specific stress indicators in honey bees.