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Review

Environment–host–parasite interactions in mass-reared insects

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The mass production of insects is rapidly expanding globally, supporting multiple industrial needs. However, parasite infections in insect mass-production systems can lower productivity and can lead to devastating losses. High rearing densities and artificial environmental conditions in mass-rearing facilities affect the insect hosts as well as their parasites. Environmental conditions such as temperature, gases, light, vibration, and ionizing radiation can affect productivity in insect mass-production facilities by altering insect development and susceptibility to parasites. This review explores the recent literature on environment–host–parasite interactions with a specific focus on mass-reared insect species. Understanding these complex interactions offers opportunities to optimise environmental conditions for the prevention of infectious diseases in mass-reared insects.

Highlights

Mass-reared insects are kept in artificial environments different from their natural habitats. Additionally, insect populations kept at high densities are generally more susceptible to parasites, which can have devastating impacts on insect mass-rearing systems.

Environmental conditions affect parasites directly and indirectly by altering insect immunity, microbiota, development, and reproduction, which are all important aspects in combatting parasites. In this way, host–parasite interactions are altered by the environment.

The environmental conditions in mass-rearing systems can often be precisely controlled. Optimising environmental conditions in insect rearing is therefore a promising tool to reduce the risks caused by parasites in combination with existing hygiene practices.

Parasites and environmental stress in mass-reared insects

For thousands of years, humans have mass-reared domesticated insects such as honeybees and silkworms. The mass rearing of insects on an industrial scale, however, is a relatively new concept, and this burgeoning industry is vital in producing insects for research, pollination services, and biological control of pests and vectors [1,2]. The most recent development is the production of insects, such as flies, mealworms, crickets, and locusts, as a protein source for aquaculture, livestock, and human consumption [1,3,4].

A key threat to insect rearing is the risk of infection by parasites (we use the word 'parasite' to refer collectively to microbial pathogens, macroparasites, and parasitoids). Parasites might be present as covert infections in mass-reared insect populations [5] or they might be introduced via the feed, addition of insect stocks, the air, or wild insects [6]. High prevalence and transmission of parasites are more likely in mass culture than in natural populations as insects are reared at very high densities [3]. Parasites can cause lethal or sublethal effects leading to substantial economic losses [3,7–11] (Box 1).

Mass-reared insects are also exposed to a range of abiotic environmental stressors. High insect densities can lead to elevated temperatures due to **metabolic heat production** (see Glossary), and this may be exacerbated by low air exchange [12–14]. In addition, high insect densities lead to accumulation of carbon dioxide (CO_2) [15–17] and other gases [17] due to respiration. Moreover, relative humidity and moisture content might be increased when insects are kept at high densities [5]. Certain insects (e.g., dipteran, hymenopteran, or orthopteran species) require a supplementary controlled lighting supply during the day [18–20], which can become stressful if the intensity or duration of light exposure are unsuitable. Rearing processes often include transport, handling, and sieving of insects, which result in mechanical vibrations [21]. In addition, ionising radiation is used in the **sterile insect technique (SIT)** which is employed to control insect pests and vectors of human diseases [1,22,23].

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Box 1. Examples of economic losses caused by parasites in mass-reared insects

- Several parasites have caused losses in silkworm production systems since their establishment more than 6000 years ago in China [10]. In more recent times, the largest economic impact on the silkworm industry was caused by the microsporidium *Nosema bombycis* causing the highly lethal disease 'pébrine' in the domestic silkworm (*Bombyx mori*) [90]. *N. bombycis* was first described in 1857, and in 1865 it caused the annihilation of the French and Italian silkworm industries [9,10]. To this day, *N. bombycis* is considered to be the main risk in silkworm production systems [90,91]. The severe impact of this parasite has led to the implementation of hygienic measures and the keeping of eggs and early instars in specialised well-equipped facilities to reduce the risk of infection [10].
- The mite *Varroa destructor* is considered to be the parasite with the biggest economic impact on colonies of the Western honeybee (*Apis mellifera*) [64]. This parasite suppresses its host's immune system, and is a vector of several viruses such as deformed wing virus (DWV) [92]. *V. destructor* came to Europe in the 1970s, and in the 1980s it was found in the USA [93]. Together with other interacting factors, such as pesticides, climate change, and other parasites, *V. destructor* is likely one of the major causes of colony collapse disorder (CCD) [92,94]. A variety of methods are nowadays used to control *V. destructor* ranging from the application of chemical treatments to hygienic practices [10].
- *Acheta domesticus* densovirus (AdDV) can severely affect mass-reared house crickets (*A. domesticus*). This virus was first identified in 1977 in a Swiss mass-rearing facility of *A. domesticus* [95] and has frequently led to devastating epidemics in European mass-rearing facilities [7,11], forcing many producers to discontinue *A. domesticus* production [11]. Since 2009, severe outbreaks of AdDV have also been recorded in Northern America, with losses of hundreds of millions of dollars in the production of *A. domesticus* as pet feed [7]. It is suggested that the virulence of AdDV is increased when the crickets are exposed to other stressors such as crowding, high relative humidity, or temperatures above 35°C [11].

The susceptibility of insect hosts and the virulence of their parasites depend on, and may be altered by, environmental conditions. Because hosts and parasites often have different environmental conditions at which performance is maximised, the impacts of the environment on host–parasite interactions are not trivial [24]. For example, if a host and a parasite have different thermal optima, there will be a mismatch of thermal performance, making the outcome of infection dependent on temperature [24,25]. Different host traits (e.g., growth and immune response) often have different environmental performance curves [26]. In mass-reared insect cultures, these trade-offs need to be considered when optimising environmental conditions for the most relevant host traits.

It is critical to understand how the environment affects host–parasite interactions to avoid losses caused by parasites (Box 1). The effects of abiotic environmental conditions on parasites of mass-reared insects have recently been reviewed [5]. Our aim here, by contrast, is to describe the impact of abiotic environmental conditions on different aspects of the insect host performance and how that may alter the outcomes of host–parasite interactions in mass-reared insects (Figure 1). We identify implications for the mass production of insects and knowledge gaps in this area. We focus predominantly on insect species that are commonly mass reared [1,4], using literature from other insect species in some cases to give a better overview of possible interactions.

Environment and immunity

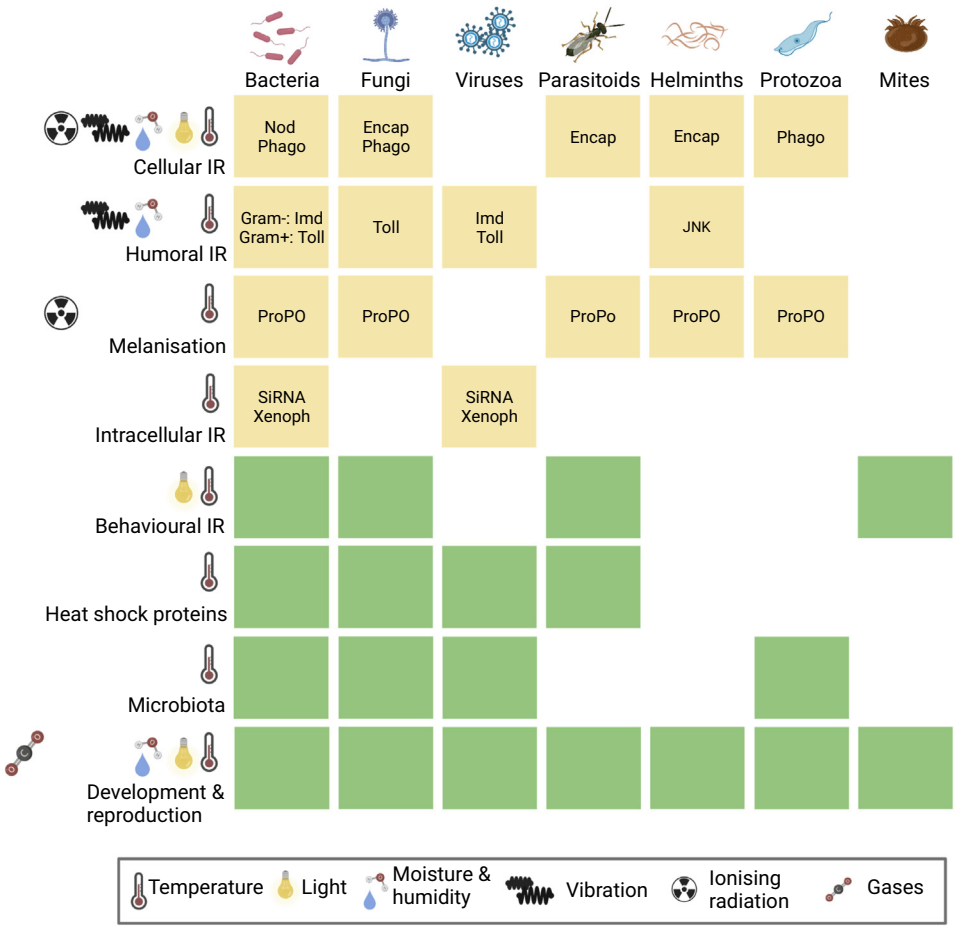
Different parts of the innate immunity and the behavioural immunity in insects act specifically against different parasite groups (Figure 1). The relationship between environmental conditions and innate immunity can be a result of crosstalk (parasite infection and environmental stress induce the same signalling pathway) or cross-tolerance (the same mechanism protects from both parasite infection and damage by environmental stress) [27]. Cross-tolerance appears to be important in mass-reared insects in relation to temperature as outlined in the following examples.

Effects of temperature on innate immunity

Temperature is the environmental condition most frequently studied in relation to insect immunity (Table 1 and Figure 1). A temperature change can increase or decrease the insect's innate immune

Glossary

Antimicrobial peptide (AMP): a heterogeneous group of short-chained amino acids involved in the insect humoral immune response active against a broad range of parasites.
Blastospores: asexual fungal spores formed from hyphae inside the insect host during the infection process.
Diapause: the period in which insect development is delayed due to adverse environmental conditions, such as cold temperatures during winter.
Ectotherm: an organism that depends on the environmental conditions to regulate its body temperature.
Haemocytes: cells involved in the immune response of insects. Haemocyte concentrations can increase in response to infection in order to encapsulate, phagocytose, or lyse parasites.
Haemolymph: fluid in invertebrates analogous to blood in vertebrates. It contains and transports haemocytes, nutrients, and other compounds.
Heat shock proteins (HSPs): a family of proteins expressed after stress to protect denaturation of polypeptides or helping other proteins to refold.
Host microbiota: the community of microorganisms that exists inside or on the host.
Melanisation: a process involved in parasite encapsulation, formation of cytotoxic components, and wound healing, which results in dark pigmentation of melanised areas.
Metabolic heat production: generation of heat due to physiological processes of the insects.
Phenoloxidase: a key enzyme in the cascade leading to melanisation. Phenoloxidases occur in the haemolymph as prophenoloxidases before they are activated.
Phototaxis: movement of an organism towards a light source.
RNAi: a process in eukaryotic cells in which double-stranded RNA molecules suppress mRNA either for host gene regulation or as a defence mechanism against parasites.
Sterile insect technique (SIT): a method in which mass-reared males of an insect species are treated with ionizing radiation and thereby sterilised. The sterile males are thereafter released in massive numbers to mate with wild females, which then cannot produce offspring.



Symbiont: one partner in an intimate ecological relationship (symbiosis). The relationship can be either mutualistic, commensal, or parasitic.

Figure 1. Possible effects of the environment on host–parasite interactions. Different parasites are known to interact with innate immune responses (shown as yellow boxes) and other components (shown as green boxes) of mass-reared insects. Conversely, environmental stressors (left panel, explained in legend) are known to affect the insect hosts, and they can thereby affect the infection outcome. The cellular immune response involves different types of haemocyte. The differentiated haemocytes are formed from stem cells called prohaemocytes, which are released into the haemolymph [68]. Smaller targets can be engulfed by single haemocytes through phagocytosis (Phago). For various bacteria, several haemocytes form nodules (Nod) surrounding the targets, and for larger parasites this process is called encapsulation (Encap). The humoral immune response involves the production of antimicrobial peptides. Different parasites trigger different signal transduction pathways – immunodeficiency (Imd), Toll, and c-Jun-N-terminal kinase (JNK) pathways [99,100]. The cellular and humoral response are both involved in the melanisation process, which is initiated by the activation of the prophenoloxidase (ProPO) pathway to produce phenoloxidase. As a result, the dark pigmented melanin is produced surrounding a parasite in the encapsulation process or around a wound [68,100]. Intracellular immune responses, such as RNAi and xenophagy (Xenoph), are active against viruses and intracellular bacteria [100]. RNAi is regulated by the siRNA pathway [99] and it silences essential parasite genes by producing small RNA sequences by the host, which interfere with the parasite RNA [100].

response, which often correlates with altered susceptibilities to parasites (Table 1). Temperature stress may also have transgenerational effects. Greater wax moth (*Galleria mellonella*) larvae in **diapause** induced by low temperature, for example, had a reduced encapsulation rate in the **haemolymph**, which coincided with a decreased survival probability when exposed to the fungal parasite *Cordyceps militaris* [28]. By contrast, elevated temperature frequently increases innate immune responses, such as **antimicrobial peptide (AMP)** production, increasing survival probabilities of *G. mellonella* larvae when exposed to parasites [29,30]. Interestingly, temperature stress can also induce transgenerational changes in innate immune responses; cold stress applied to the parental

Table 1. Overview of recent studies focusing on the effects of environmental stress on the innate immune response and the impact on susceptibility to parasites of mass-reared insect species[a]

Environmental stress	Host species	Host sex and life stage	Parasite species	Stress increases (↑), decreases (↓), or does not affect (↔) immune response	Stress increases (↑), decreases (↓), or does not affect (↔) susceptibility (i.e., susceptibility to death unless otherwise stated) to parasites	Refs
Elevated temperature	*Drosophila melanogaster* (common fruit fly)	Female and male larvae and pupae	*Leptopilina boulardi* (parasitoid wasp) strain ISm	↔ Encapsulation	Susceptibility to becoming parasitised: ↔	[67]
			L. boulardi strain ISy	↓ Encapsulation	Susceptibility to becoming parasitised: ↔	[67]
		Female and male adults	*Pseudomonas aeruginosa* (bacterium)	↓ Cuticular melanisation	↑	[48]
		Female adults	*P. aeruginosa*	Expression of immune response genes (↔ Pgrp-LC, ↔ relish, ↔ diptericin)	↔	[65]
			Lactococcus lactis (bacterium)	Expression of immune response genes (↓ spatzle, ↔ cactus, ↔ metchnikowin)	↔	[65]
	Galleria mellonella (greater wax moth)	Female and male larvae	*Candida albicans* (fungus)	↑ Haemocyte conc., ↑ AMP gene expression (gallerimycin, transferrin, inducible metalloproteinase inhibitor, galiomicin)	↓	[29]
			Malassezia furfur (fungus)	↓ Melanisation	↔	[96]
			Metarhizium robertsii (fungus)	↑ Lysozyme-like activity, ↑ PO, ↔ encapsulation, ↓ AMP gene expression (galiomicin and gallerimycin)	↓	[36]
			Streptococcus agalactiae (bacterium)	↔ Cuticular melanisation	↔	[97]
	Megachile rotundata (alfalfa leafcutting bee)	Female and male larvae	*Ascosphaera aggregata* (fungus)	↑ Overall expression of immune response genes	↓	[19]
Reduced temperature	*D. melanogaster*	Female and male larvae and pupae	*L. boulardi* strain ISm	↔ Encapsulation	Susceptibility to becoming parasitised: ↔	[67]
			L. boulardi strain ISy	↑ Encapsulation	Susceptibility to becoming parasitised: ↓	[67]
		Female adults	*P. aeruginosa*	Expression of immune response genes (↑ Pgrp-LC, ↔ relish, ↔ diptericin)	↔	[65]
			L. lactis	Expression of immune response genes (↔ spatzle, ↔ cactus, ↔ metchnikowin)	↔	[65]
			Metarhizium anisopliae (fungus)	↔ Haemocyte conc., ↔ PO, expression of immune response genes (↔ drosomycin, ↔ defensin,↔ diptericin, ↑ Turandot-A, ↔ cecropin, ↔ metchnikowin, ↔ drosocin, ↔ vir-1), ↔ wound-induced melanisation	↔	[98]

(continued on next page)

Table 1. (continued)

Environmental stress	Host species	Host sex and life stage	Parasite species	Stress increases (↑), decreases (↓), or does not affect (↔) immune response	Stress increases (↑), decreases (↓), or does not affect (↔) susceptibility (i.e., susceptibility to death unless otherwise stated) to parasites	Refs
	G. mellonella	Female and male larvae	*Aspergillus fumigatus* (fungus)	24, 48, or 72 h after stress: ↓↔↔ haemocyte conc., expression of immune response genes (↔↔↔ apolipophorin, ↔↔↔ arylphorin, ↔↔↔ prophenoloxidase)	↑ When exposed to parasite 24 h after stress, ↔ when exposed to parasite 72 h after stress	[54]
			Steinernema feltiae (nematode)	↓ PO, ↑ lysozyme activity	↔	[66]
			Steinernema carpocapsae (nematode)	↓ PO, ↑ lysozyme activity	↓	[66]
			Heterorhabditis bacteriophora (nematode)	↓ PO, ↑ lysozyme activity	↓	[66]
			Bacillus thuringiensis (bacterium)	↓ PO, ↑ lysozyme activity	↑	[66]
			C. albicans	↑ Haemocyte conc., ↑ AMP gene expression (gallerimycin, transferrin, inducible metalloproteinase inhibitor, galiomicin)	↓	[29]
			Cordyceps militaris (fungus)	↓ Antifungal peptide gene expression, ↑ antibacterial peptide gene expression	↑	[73]
			C. militaris	↓ Encapsulation, ↔↓ PO (depending on temp.)	↑	[28]
	M. rotundata	Female and male larvae	*A. aggregata*	↑ Overall expression of immune response genes	↓	[19]
Short (<2 h) elevated temperature	*G. mellonella*	Female and male larvae	*B. thuringiensis*	↑ Antimicrobial activity of larval haemolymph, ↑ expression of gallerimycin, cecropin and galiomicin in the fat body, ↔ expression of the metalloproteinase inhibitor-IMPI	↓	[30]
			Beauveria bassiana (fungus)	↓ Expression of gallerimycin and galiomicin, ↑ lysozyme-like activity, ↑ antifungal activity of haemolymph	↓	[87]
	Tribolium castaneum (red flour beetle)	Female and male adults (exposed to stress), adult offspring (effects measured)	*B. thuringiensis*	↓ PO	↔	[31]
Short (<2 h) reduced temperature	*D. melanogaster*	Female adults	*M. anisopliae*	↑ Haemocyte conc., ↔ PO, expression of immune response genes (↔ drosomycin, ↔ defensin,↑ diptericin, ↑ Turandot-A, ↔ cecropin, ↔ metchnikowin, ↔ drosocin, ↔ vir-1), ↔ wound-induced melanisation	↔	[98]

Table 1. (continued)

Environmental stress	Host species	Host sex and life stage	Parasite species	Stress increases (↑), decreases (↓), or does not affect (↔) immune response	Stress increases (↑), decreases (↓), or does not affect (↔) susceptibility (i.e., susceptibility to death unless otherwise stated) to parasites	Refs
	T. castaneum	Female and male adults (exposed to stress), adult offspring (effects measured)	*B. thuringiensis*	↑ PO	↓	[31]
Fluctuating temperature	*Apis mellifera* (Western honeybee)	Female pupae and adults	*M. anisopliae*	↑ PO	↑	[34]
Vibration	*G. mellonella*	Female and male larvae	*A. fumigatus*	24, 48, or 72 h after stress: ↑↑↔ haemocyte conc., expression of immune response genes (↑↔↔ apolipophorin, ↑↑↔ arylphorin, ↔↔↔ prophenoloxidase)	↓ When exposed to parasite 24 h after stress, ↔ when exposed to parasite 72 h after stress	[54]
			C. albicans	↑ Haemocyte conc., ↑ AMP gene expression (galiomicin, inducible metalloproteinase inhibitor), ↔ AMP gene expression (transferrin, gallerimycin)	↓	[37]
Ionising radiation	*Ceratitis capitata* (Mediterranean fruit fly)	Female and male larvae	*Diachasmimorpha longicaudata* (parasitoid wasp)	↓ Encapsulation	↑	[18]

[a]Abbreviations: AMPs, antimicrobial peptides; conc., concentration; PO, phenoloxidase activity.

generation of red flour beetles (*Tribolium castaneum*) increased **phenoloxidase** activity in their offspring and decreased mortality of the offspring when exposed to a bacterium (*Bacillus thuringiensis*). Moreover, *T. castaneum* larvae from cold-stressed parents had an increased development time until pupation compared with larvae from parents that did not receive a cold stress, which indicates a trade-off between immune response and development [31]. The effect of temperature on intracellular immunity has been studied in mosquitoes (*Aedes aegypti*). **RNAi** was hindered in adult mosquitoes reared at 18°C compared with those reared at 28°C, which coincided with elevated infection levels of chikungunya virus (CHIKV) and yellow fever virus at low temperatures [32].

The investment in immune responses requires energy. However, energy for immune responses has been suggested to be limited when insects cope with thermal stress [33,34], and potentially other environmental stresses. Adult fruit flies (*Drosophila melanogaster*) kept at 18°C, as opposed to 25°C, downregulated the expression of AMP genes. By inducing downregulation in mutant flies independently of temperature, the authors demonstrated that AMP downregulation led to prolonged lifespans and augmented stress resistance, for example, in the case of starvation [35]. Thermal stress can also have the opposite effect, resulting in increased immunity [19,29,30,36,37]. One possible mechanism for this phenomenon is cross-tolerance. Tang *et al.* [38] found that the expression of **heat shock proteins (HSPs)** in housefly (*Musca domestica*) larvae increases after heat stress but also after bacterial challenge (*Escherichia coli* or *Staphylococcus aureus*). Interestingly, a lack of these HSPs (due to silencing of HSP gene expression using RNAi) then led to lower survival after bacterial infection or heat stress, which

proves that the same mechanism protects from heat stress and infection [38]. Similarly, HSP gene expression has been shown to increase after exposure of *D. melanogaster* to RNA viruses [Drosophila C virus (DCV), Cricket paralysis virus (CrPV), or Invertebrate iridescent virus (IIV-6)] [39]. HSPs are traditionally not considered as part of innate immunity in insects. Nevertheless, we know from other invertebrates that HSPs can enhance phagocytosis, increase phenoloxidase production, and protect host protein denaturation during parasite infection [40]. An upregulation of HSP gene expression after infection is thought to be beneficial to the host in silkworm (*Bombyx mori*) eggs parasitised by a parasitoid wasp (*Telenomus theophilae*) [41] or in *G. mellonella* infected with the fungus *Conidiobolus coronatus* [42]. In *B. mori* cells, however, HSPs support the proliferation of *B. mori* nucleopolyhedrovirus (BmNPV) [43,44], demonstrating that the role of HSPs is not always beneficial to the insect host.

Temperature might also affect insect immunity through its effect on the insect cuticle. Yellow mealworm (*Tenebrio molitor*) larvae reared at 28°C had darker cuticles (higher degrees of cuticular **melanisation**) as adults, compared with adults that had been reared at lower temperatures (18°C or 23°C) during their larval stage [45]. In addition, darker beetles also had an increased survival probability compared with lighter beetles when exposed to a fungal parasite (*Metarhizium anisopliae*) [46]. However, it is unclear if this was a result of decreased ability of the fungus to penetrate highly melanised cuticles or if it was because increased melanisation correlates with other innate immune responses [47], leading to the lower mortality in beetles exposed to *M. anisopliae* [46]. In adult *D. melanogaster,* darker cuticles correlated with increased survival when exposed to a bacterium (*Pseudomonas aeruginosa*) [48], a parasite that does not infect the host by penetrating the cuticle. Here, on the contrary, higher temperatures led to lighter cuticles [48]. Melanisation as a result of temperature change possibly demonstrates cross-tolerance [48], as cuticular melanisation is known to play a role in thermoregulation [49] but also in immunity.

Effects of ionising radiation, humidity, light, and vibration on innate immunity

In contrast to our knowledge of the effect of temperature, our understanding of the effects of other environmental conditions on innate immunity in mass-produced insects is limited. The effect of ionising radiation on the immune response has been studied in Mediterranean fruit flies (*Ceratitis capitata*), which are commonly reared for SIT. In contrast to control larvae, irradiated larvae (40 gray – the SI unit of ionising radiation dose) did not accumulate phenoloxidase over the course of development [22]. Similarly, encapsulation rates and adult emergence decreased in *C. capitata* with increasing ionising radiation when parasitised by a parasitoid wasp (*Diachasmimorpha longicaudata*) [18]. Ionising radiation is thought to affect the cellular immune response of insect hosts directly; as **haemocytes** lack pigmentation, they are thought to be sensitive to this type of radiation [23]. This highlights the need to optimise the ionising radiation dose, taking into account sterility, fitness, and other traits.

Humidity affects parasite survival outside the host as well as both parasite virulence [5,50] and host susceptibility in insects. For example, in larvae of the lepidopteran pest species beet armyworm (*Spodoptera exigua*), increasing relative humidity led to decreased antioxidant activities, which correlated with decreased survival when exposed to a nucleopolyhedrovirus (SeNPV) [51]. Conversely, the larvae of the Mediterranean flour moth (*Ephestia kuehniella*) showed increased nodulation at an elevated relative humidity (85%) when infected with *B. thuringiensis* compared with larvae kept at a low relative humidity (43%) [52]. However, the effects of humidity on the immune responses of mass-reared insects remain to be investigated more thoroughly.

The effects of artificial light have been studied in the Australian black field cricket (*Teleogryllus commodus*), which is not commonly mass-reared but used as a model organism. Durrant *et al.* [53] found that dim artificial light during the night decreased haemocyte concentration in the

haemolymph, which could have negative effects on infection outcome in case of an exposure to parasites [53].

In contrast to the decreased immune responses observed in response to ionising radiation and light, short periods of vibration have been shown to increase haemocyte concentrations and the expression of several AMP genes in *G. mellonella* larvae, coinciding with lowered mortalities when exposed to the fungal parasites *Candida albicans* [37] and *Aspergillus fumigatus* [54].

Effects of the environment on behavioural immunity

Environmental conditions are also of high relevance in behavioural immunity (host behaviours that lead to the avoidance or mitigation of parasite infection [55,56]). When given the choice, certain insect species increase their body temperature by seeking places with elevated temperatures to suppress the development of parasites [20,56–58], a phenomenon called behavioural fever or fevering. Adult migratory locusts (*Locusta migratoria*) infected with *M. anisopliae*, for example, had an 85% higher survival rate when given the opportunity to increase their body temperature for at least 4 h per day compared with adults that were not enabled to fever [56]. The reduced mortality rate due to behavioural fever is potentially a combined effect of parasite inhibition at elevated temperatures and the triggering of the immune response of the host (Figure 1). Sangbaramou *et al.* [20] found that *L. migratoria* nymphs exposed to the fungal parasite *Beauveria bassiana* had an elevated haemocyte concentration and an absence of circulating fungal **blastospores** in their haemolymph when given the opportunity to increase their body temperature behaviourally. By contrast, nymphs kept at constant temperatures had fewer haemocytes, and circulating fungal blastospores were observed. This correlated with higher mortality in nymphs kept at constant temperatures compared with fevering nymphs [20].

It is important to note that behavioural fever is not exhibited against all parasite species. Adamo *et al.* [59] found that house crickets (*Acheta domesticus*) increased their body temperature when infected with the Gram-negative bacterium *Rickettsiella grylli*, leading to increased survival. However, when the crickets were infected with another Gram-negative bacterium (*Serratia marcescens*), they did not increase their body temperature. Moreover, increasing the temperature artificially did not increase survival when exposed to *S. marcescens*, demonstrating that behavioural fever can be targeted by the insects against parasites that are affected by the temperature change (i.e., *R. grylli*) but not against parasites that remain unaffected by the temperature increase (i.e., *S. marcescens*) [59]. In adults of *M. domestica*, the intensity of the behavioural fever was found to be positively correlated with the dose of *B. bassiana* they received [60], indicating that insects can optimise the costs and advantages of behavioural fever.

In contrast to increasing the body temperature by fevering, buff-tailed bumblebees (*Bombus terrestris*) actively lower their body temperature by remaining in the field overnight when parasitised with parasitoid conopid flies to delay parasite development [61]. Similarly, adult *D. melanogaster* infected with a fungal (*Metarhizium robertsii*) or bacterial (*P. aeruginosa*) parasite exhibited a preference for cooler temperatures compared with uninfected flies; the survival probabilities of the flies when infected was increased under these cooler temperatures [62,63]. This demonstrates that the ability to raise and to lower temperature behaviourally can be beneficial for infected hosts.

A change in temperature could also act as a cue for parasitism, allowing social insects to react to infested broods. Brood cells of the Western honeybee (*Apis mellifera*) parasitised by mites (*Varroa destructor*) have a higher temperature than nonparasitised cells. Bauer *et al.* [64] therefore suggested that worker bees might use these elevated temperatures as a cue to remove infested

brood cells, a hygienic behaviour that can decrease parasite dispersal inside the beehive. The causal relationship between parasitism, elevated temperature, and brood removal however remains to be demonstrated [64]. A further form of behavioural immunity through **phototaxis** has been shown recently in adult flies (*Drosophila nigrospiracula*) being infested by mites (*Macrocheles subbadius*) whereby the risk of mite infestation is higher in the dark than in the light. Hence, flies demonstrated avoidance behaviour, spending more time in lightened areas than in dark areas when mites were present [55].

Investigating the effects of environment on immunity is complex

To deal with the complexity of the insect immune system, several immune responses can be studied simultaneously and over time [54,65] in combination with experimentally establishing the susceptibility of hosts to their parasites, as done in the studies presented in Table 1. Several factors will define how the environmental conditions affect immune responses and susceptibility to parasites of insect hosts. Different genetic strains of *C. capitata* show, for example, different parasitoid encapsulation rates [18]. Furthermore, immune responses can depend on species (Table 1) and on sex, as shown in *T. molitor* with increased encapsulation rates in females [45] and in *T. commodus* with increased haemocyte concentrations in females [53]. In social insects, the interactions between temperature and immune response are even more complex, as different castes are adapted to different environments. When worker bees, queens, and drones of *A. mellifera* were exposed to a heat stress during the pupal stage, the phenoloxidase activities in the adult stages were either increased, decreased, or remained unchanged, respectively [34]. Last, the effect of the environment on host immunity also depends on the parasite species [66], and the genetic strain of the parasite [67].

Environment, host microbiota, and parasites

Protection from parasites in insects derives not only from their own immune system, but also from their associated **host microbiota** [68], for example, by the production of antimicrobials by bacterial **symbionts** [69]. Additionally, the host microbiota can increase the ability of the host to cope with environmental stress. Adults of *D. melanogaster* exposed to the nonparasitic fungus *Aspergillus oryzae* had an increased survival probability under heat stress [70]. The parasites in turn can also affect the host microbiota. The toxins of *B. thuringiensis*, for example, can alter the gut bacterial community composition and reduce the total bacterial load in the guts of *L. migratoria* [71]. In a recent review, Savio *et al.* [72] found that members of the bacterial genus *Lactobacillus* appear to be of great importance in decreasing susceptibility of mass-reared insects to fungal and bacterial parasites, whereas members of the genera *Wolbachia* and *Spiroplasma* reduce susceptibility to viral infection [72].

Temperature affects host microbiota and thereby infection outcome

Thermal stress can act directly on parasites, but it can also affect the host's microbiota and thereby affect the outcome of infection by parasites. Studies of these interactions need to contend with complex systems and understanding of multiple interactions. For example, bacterial symbionts are suggested to have a temperature-dependent effect on the infection by a fungal parasite (*C. militaris*) in *G. mellonella* larvae. Mortality caused by *C. militaris* infection at high temperature (25°C) was reduced in comparison to lower temperature (15°C). This coincided with an increased abundance of enterococci and enterobacteria (both of which have inhibitory effects on *C. militaris in vitro*) in the haemolymph and in the gut at high temperatures in response to infection [73]. In addition, the host responses to different parasites were also temperature dependent. Expressions of lysozyme genes, which play a role in the Toll and prophenoloxidase pathways mainly active against bacteria and fungi, were increased. However, the expressions of cecropin genes, which play a role in the Imd pathway against Gram-negative bacteria (Figure 1), were decreased

at high temperatures potentially favouring the Gram-negative enterobacteria [73]. Similarly, in common Eastern bumblebees (*Bombus impatiens*), increasing temperature leads to decreased infection intensity of a trypanosomatid parasite (*Crithidia bombi*) [74]. At high temperatures (above 32°C), this can be explained by a direct growth inhibition of *C. bombi* [75]. However, at temperatures below 32°C the parasite is not directly inhibited by temperature and indirect inhibition might stem from acid-producing bacteria [74] (many bacterial gut symbionts of bees transform carbohydrates into short-chained fatty acids, acidifying the gut [76]), which increase their metabolic rates with increasing temperatures [75]. However, the relationship between temperature, host microbiota, and gut pH remains poorly understood [74]. These examples demonstrate that different infection outcomes in response to temperature may result from multiple interactions.

Environment, host development, reproduction, and parasites

Two of the most important parameters in the mass production of insects are development and reproduction, which define the productivity of the systems. As **ectotherms**, the physiology of insects is directly linked to the environmental temperature. Altered development rates due to environmental conditions might lead to a trade-off with immunity [31] as discussed before. However, highest growth rates under conditions optimised for growth, may lead to lowest susceptibility to infection because the parasite dose per host mass decreases with increasing body mass. For example, larger larvae of *G. mellonella* showed decreased mortality when exposed to the bacterium *S. aureus*, which correlated with increased lipid weights in larger larvae [77]. A similar effect might occur when optimising other environmental conditions for higher growth rates. For example, *T. molitor* larvae show increased growth rates when kept in darkness versus alternating light/dark conditions [78] and grow faster with increasing relative air humidity [79]. Larger *T. molitor* larvae show higher survival probabilities than smaller larvae when exposed to *B. thuringiensis* [80], yet the causal relationship between environment, insect body mass, and parasite susceptibility remains to be investigated.

The interaction between reproduction and immunity of the insect host when affected by the environment can lead to different outcomes. First, different energy investments under altered environmental conditions can lead to trade-offs between reproduction and immunity. In brown-banded cockroaches (*Supella longipalpa*) parasitised by an acanthocephalan parasite (*Moniliformis moniliformis*), for example, elevated temperatures led to decreased reproduction compared with healthy cockroaches, whereas at lower temperatures both parasitised and healthy cockroaches had similar reproductive outputs [81]. One possible explanation might be that the immune response is increased at elevated temperature (e.g., in response to increased parasite performance), which leaves less energy for reproduction. Second, reproduction and immunity may both be increased or decreased under environmental stress. *B. impatiens* queens exposed to a short CO_2 narcosis showed increased reproduction and at the same time an increased ability to eliminate bacteria (*Providencia rettgeri*) in their haemolymph [82], which may result in trade-offs with other energy-consuming processes that were not measured. Finally, trade-offs between immunity and reproduction depend on the parasite species. In Texan field crickets (*Gryllus texensis*), differing responses to temperature were shown when infected by different parasites; control crickets kept 7°C above average field temperature showed higher reproduction, as did crickets exposed to a sublethal dose of the Gram-negative bacterium *S. marcescens*. By contrast, when exposed to a sublethal dose of the Gram-positive bacterium *Bacillus cereus*, the elevated temperatures did not lead to higher reproduction [83]. This could not be explained by different thermal optima of the parasites, but the authors suggest that the immune response against *B. cereus* was more energy intensive than that against *S. marcescens,* leading to lower investment in reproduction [83]. These examples demonstrate that the effects of environmental conditions on potential trade-offs between immunity and reproduction and

other important energy-consuming processes need to be carefully assessed before choosing environmental rearing conditions (Figure 2).

Implications and applications for mass rearing of insects

Mass-rearing conditions are set to maximise productivity (i.e., output of nondiseased insects). However, parasitic infections can lead to devastating losses in production systems. Environmental conditions play an essential role in the defences of mass-reared insects to parasites (Figure 1 and Table 1). Adjusting these conditions to increase defences against parasites should therefore be considered when choosing environmental conditions (Figure 2), which will in turn lower the risk of lethal and sublethal effects caused by parasites and maintain productivity of insect mass rearing.

The environmental conditions can be constant or fluctuating throughout the mass-rearing process. Extreme environmental conditions can also appear as 'shocks' (i.e., short-term changes

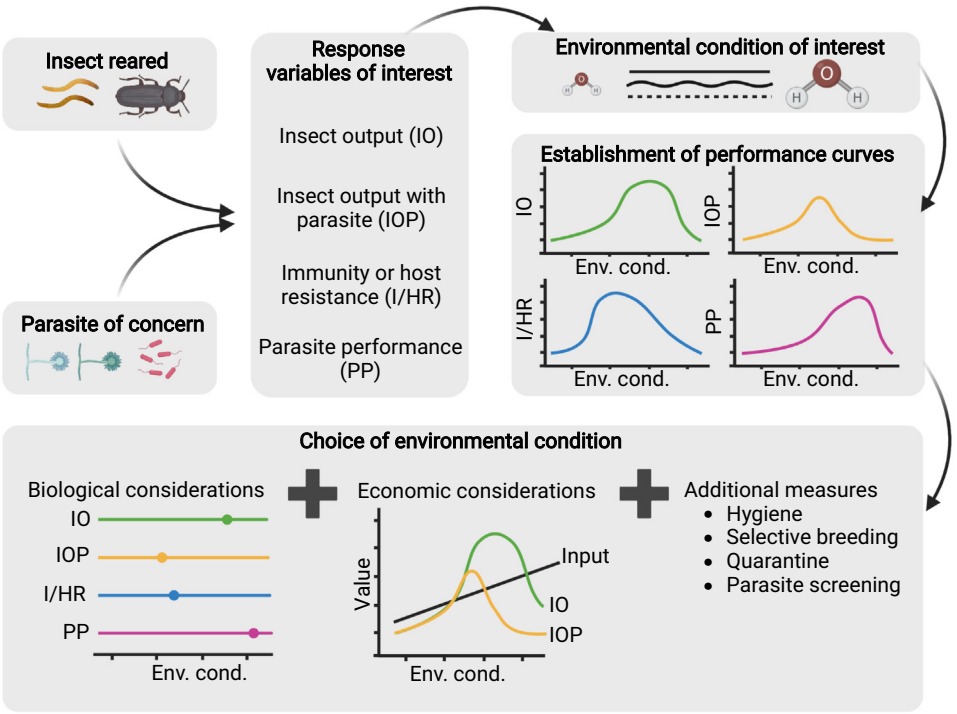


Figure 2. Workflow for choosing environmental conditions in mass-rearing systems. The insect (species, strain, and life stage) reared and the parasite (species and strain) of concern define the response variables to be measured. The response variables should at least consist of a measure of insect output (e.g., insect biomass) when the population is parasite-free and when parasitised, a measure of host resistance or immunity (e.g., encapsulation), and a measure of parasite performance (e.g., germination of fungal spores). Thereafter, the environmental condition of interest is chosen (e.g., humidity is illustrated here). Environmental conditions can remain constant over time (continuous line), occur as fluctuations (wavy line), or as shocks (broken line). The chosen response variables are measured over a range of levels of the environmental condition to establish performance curves (shown as hypothetical curves). Finally, choosing the environmental condition is based on biological and economic considerations, and additional measures that are feasible. Different response variables potentially have different peak performances in response to environmental conditions, which allows choosing a condition that is furthest away from peak parasite performance but closest to maximised insect immunity and output. For the economic considerations, the risk of parasite presence needs to be assessed. In a situation of low parasite infection risk (influenced by additional measures), maximised insect output (green curve) and monetary input value are used for calculating the optimal environmental condition. In a situation of high parasite infection risk (e.g., because of costly additional measures), maximised insect output with parasite infection (yellow curve) and input value are used to identify the optimal environmental condition.

of conditions) (Figure 2). These different regimes of environmental conditions can either be a result of intentional measures to increase productivity or they can be a result of the rearing process itself. Fluctuating temperatures in an innocuous thermal range are often beneficial for a variety of different response variables of insects (e.g., reproduction, growth, thermal tolerance, and development) [26]. Spring field crickets (*Gryllus veletis*) that were acclimatised to fluctuating temperatures had higher survival probabilities when infected with the fungal parasite *Metarhizium brunneum* compared with crickets kept at constant temperatures [25]. Similarly, *T. molitor* larvae had increased antibacterial activity in their haemolymph when exposed to a fluctuating temperature regime (±8°C) compared with larvae kept at constant temperatures [84]. Interestingly, fluctuating temperatures have also been found to mediate the course of coinfections. The fungus *M. anisopliae* is highly virulent to desert locusts (*Schistocerca gregaria*) under constant temperatures (30°C). However, under a fluctuating temperature regime (20–42°C) the survival of *S. gregaria* infected with *M. anisopliae* decreased only when they were additionally exposed to another fungus (*B. bassiana*). This is interesting as *B. bassiana* on its own did not decrease survival under fluctuating temperatures compared with uninfected locusts [85]. In certain systems, the outcomes of host–parasite interactions under fluctuating temperatures can be predicted by calculating the averages of the outcomes at constant maximal and minimal temperatures [25], although this is not always possible in other systems [86].

Insects kept at high densities produce metabolic heat leading to temperature gradients in rearing containers, with the highest temperatures in the centre, compared with the lowest temperatures at the edges [12–14]. Such temperature gradients provide the opportunity for mass-reared insects (i.e., insects of the orders Orthoptera, Diptera, and Hymenoptera) to exhibit behavioural fever or cooling that may lead to avoidance or suppression of parasites. To our knowledge, there have been no studies to date that explore temperature selection in response to parasite infection in mass-rearing settings.

Short thermal or physical shocks can occur during handling of mass-reared insects, such as transportation or during sieving processes. These shocks had beneficial effects on the host immunity and survival when exposed to parasites [29,30,37,87]. Browne *et al.* [54] found that the beneficial effects of thermal and physical shocks peak 24 h and diminish 72 h after the stress [54], which indicates that these short exposures might not be useful to increase immunity over a prolonged period. The regulation of immune response gene expression depends on duration and frequency of thermal shocks [88]. However, the frequent applications of thermal and physical shocks on the immune response and susceptibility of mass-reared insect species have, to our knowledge, not been tested thus far. Such studies would be essential to understand the long-term effects and potential trade-offs in reproduction, growth, or other traits. Furthermore, transgenerational trade-offs between immune response and development are possible [31] and should be considered in the context of using environmental stress to decrease offspring susceptibility to parasites.

In order to make informed decisions regarding choice of environmental conditions, performance curves of relevant response variables are needed. In addition to biological considerations, economic considerations need to be taken into account in any mass-rearing system that is commercially producing insects [26]. Depending on the degree of risk of parasite infection, the chosen environmental conditions may differ to mitigate against infection, whilst maintaining biological and economic optimal outcomes (Figure 2).

Concluding remarks

Environmental conditions affect host–parasite interactions in mass-reared insects directly or indirectly by changing immunity, microbiota, development, and reproduction of the insect hosts. Optimising environmental conditions merely for increased production (i.e., growth and reproduction)

Outstanding questions

Does increased investment into immunity following environmental stress lead to negative impacts in mass-produced insects?

How do coinfections alter host and parasite performance under different environmental conditions? What are the implications for mass-rearing insects?

Can mismatches in parasite and host performance under different environmental conditions (specifically relative humidity, moisture content, and gas concentrations) be utilised to alter infection outcomes in mass-reared insects?

may lead to higher susceptibility to parasites as energy investment into a certain trait (e.g., reproduction) can reduce energy investment into another trait (e.g., immunity) [83]. However, environmental conditions can also be optimised to reduce the risk of parasite infection in mass-rearing systems (Figure 2) whilst maintaining an adequate level of insect quality.

Although our knowledge of how the environment affects host–parasite interactions in mass-reared insect species has expanded recently, several key questions remain (see Outstanding questions). Additional efforts are needed to understand sublethal effects (e.g., effects on weight gain or reproductive output) of environment–host–parasite interactions, as they have a tremendous potential to reduce productivity in the mass production of insects in the long term. Moreover, efforts are needed to investigate key mass-reared insect species as well as the parasite species that are challenges in mass-production systems, as our current knowledge stems from a few model organisms (Table 1). We also need to acknowledge that the environment in which insects are reared is a combination of both differing environmental conditions and potentially multiple parasites infecting hosts simultaneously. Future research should therefore consider how different environmental stressors and parasites interact with each other, as the outcomes of such interactions might not be predictable by studying the stressors or parasites individually [85,89]. There remains a significant dearth of knowledge on how moisture content, gas concentrations, and relative humidity affect host–parasite interactions in mass-reared insect species and how the host microbiota is affected by different environmental conditions. Finally, it should be considered that optimising environmental conditions is one of many options available to maintain insect health in mass-reared systems. The simultaneous use of other interventions and tools (Figure 2) will all help to keep parasites under control in insect mass-rearing systems.

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Declaration of interests

The authors declare no competing interests.

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