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Farmed yellow mealworm (*Tenebrio molitor*; Coleoptera: Tenebrionidae) welfare: species-specific recommendations for a global industry

M. Barrett[1,2], R.K. Godfrey[2,3], A. Schnell[2,4] and B. Fischer[2,5]*

[1]Department of Biology, California State University Dominguez Hills, Carson, CA 90747, USA; [2]Rethink Priorities, 530 Divisadero St, San Francisco, CA 94117, USA; [3]Florida Museum of Natural History, University of Florida, 3215 Hull Rd, Gainesville, FL 32611, USA; [4]Department of Psychology, University of Cambridge, Cambridge, CB2 3EB, UK; [5]Department of Philosophy, Texas State University, 601 University Dr, San Marcos, TX 78666, USA; [*]meghan.barrett21@gmail.com

Supplementary material

FILE S1 This contains the full review of all biological information reviewed for yellow mealworms, organized by topic.

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Nutrition

Larval nutrition

Nutrition impacts development and survival in YML (Oonincx et al. 2015). Wheat bran (13-18% protein, 56% carbohydrates; Prückler et al. 2014) is the most common component of larval diets, containing all the necessary nutrients for YML growth and survival though the nutrient proportions are not optimal (Ribiero et al., 2018, Morales-Ramos et al., 2010). Indeed, feeding on wheat bran-only diets results in significantly longer developmental time and lower survival rates (Morales-Ramos et al. 2010). Despite this, feeding wheat bran-only diets may be common practice in the industry (Deruytter et al. 2020).

YML have increased survival on a heterogenous diet of cereal (e.g., wheat bran), a dedicated source of protein (e.g., soy protein, beer yeast) and a water source (often vegetables, e.g., potato; see section 'Hydration'). Providing a source of protein improves pupal weight gain, reduces development time, and significantly increases larval survival rates (van Broekhoven et al. 2015; Oonincx et al. 2015; Morales-Ramos et al. 2013; Figure 1). Sources of protein in the larval stage can also improve adult fecundity, with egg production increasing from 3-4 eggs/d to 6–7 eggs/d. Larvae preferred 20% egg protein diets over diets of soy protein, or diets that mixed peanut/salmon/canola oil with soy and egg protein (Morales-Ramos et al. 2013); however, the study did not test yeast, which is the most commonly reported source of protein provided for YML (Ribiero et al. 2018). Overall, protein sources appear beneficial for YML welfare.

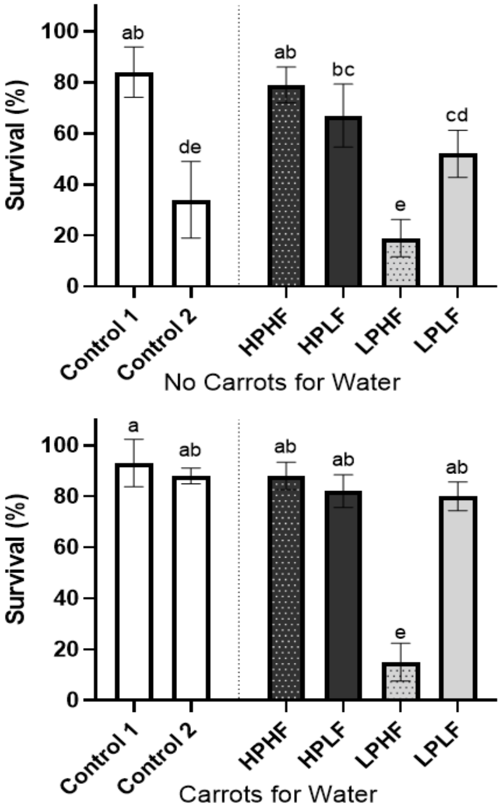

Fig 1 (left). Effects of high/low protein and high/low fat diets on YML survival, with and without water sources (carrots). Data re-plotted from Table 3 in Oonincx et al. 2015; control diets 1 and 2 are not described, but are standard formulations obtained from two industry producers. HP = high protein; LP = low protein; HF = high fat; HL = low fat.

Macronutrients can have synergistic or antagonistic interactions: for instance, high fat diets performed the best for survival when combined with high amounts of protein, but the worst when combined with low amounts of protein (Figure 3). It is possible strain (or other variables) could also influence the effects of diet of YML survival: two control diets provided by two different industrial rearing facilities performed significantly differently when fed to the same laboratory strain without a water source (34% v. 84% survival). However, when carrots were added as a water source, the strains performed statistically similarly (88% v. 93% survival). It seems unlikely that one of these producers is regularly accepting only 34% survival in their facility, and more likely that a combination of rearing conditions (e.g., water availability), diet, and/or strain differences impacted larval survival using 'control 2' in the laboratory trial. This example highlights the importance of producers monitoring the welfare of their own population and adjusting nutrition to best meet their specific insect populations' needs, considering the complex matrix of effects represented by diet, strain, and abiotic conditions.

YML are capable of nutrient self-selection to regulate their protein and carbohydrate intake, preferentially consuming a 4:1 ratio of wheat bran: dry potato flakes. This ratio provided the shortest development time but the second best ratio for population growth (Morales-Ramos et al. 2011). Nutrient ratio can also affect behaviors such as locomotion activity and responsiveness to visual, olfactory, and gustatory cues emitted by foods in insects (Barton-Browne, 1993). For example, *Malacosoma disstria* caterpillars increased their exploratory behaviors on protein-deficient, carbohydrate-rich diets (Colasurdo et al. 2007); this energetically costly behavior may be a response to malnutrition as the insects seek better sources of protein to meet their nutritional needs. Nutrient deficiency may also affect YML behavior, but further research is needed.

Industry can promote YML welfare by providing a heterogenous diet with both proteins and carbohydrates; fats may also improve survival, if the appropriate proteins are available (Oonincx et al. 2015). As larvae are capable of self-regulating their macronutrient intake, with follow-on fitness benefits, producers should provide a diverse set of macronutrient components to YML, allowing them to maximize their welfare. Fats, and to a lesser extent proteins, seem to be infrequent in YML diets. If these macronutrients are beneficial to larvae, their rarity in most standard industry diets may explain higher rates of larval cannibalism, as larvae may seek fats/proteins from within conspecifics' bodies (see section 'Cannibalism'; see Randall et al. 2023). Determining if provisioning of dietary fats/proteins reduces larval cannibalism and benefits survival, growth rate, and fecundity may be an important future research direction for improving YML welfare.

No data were found on the appropriate feeding rate for YML (e.g., batch vs. progressive feeding strategies, as discussed for black soldier fly larvae in Barrett et al., 2022); this deserves further study, as macronutrient composition and food quality may change/decline over time in batch-feeding methods. In addition, small YML are less mobile than older larvae; when vegetables are provided as water and/or to increase diet heterogeneity, producers should take care to distribute those resources well throughout the feed so all larvae will be able to take advantage of them (see further discussion of this issue in 'Hydration'; Deruytter et al. 2020). Similarly, a small particle size for added proteins/fats, alongside thorough mixing of dry ingredients, will be important for making sure all larvae have access to the complete suite of provided micronutrients.

Further, feed safety should be confirmed prior to feeding YML, given the possibility of environmental contaminants of grains/cereals that can negatively affect physical health (e.g., mycotoxins or bacteria/fungi; see section 'Environmental Pollutants' and 'Microbes'). Pre-processing of substrates (e.g., heat treatment to kill pathogens, or mixing contaminated and uncontaminated substrates to reduce toxin levels) may be necessary to make sure substrates are safe for YML consumption.

Finally, YML may be fed plastics/polymers, including polystyrene, polyethylene, poly vinyl chloride, polyester polyurethane, vulcanized natural latex rubber (tire crumb), styrofoam, and vulcanized butadiene-styrene elastomer (reviewed in Khan et al. 2021; Yang et al. 2018). It is unlikely that insects to be used as food and feed will be reared primarily on these substrates given concerns with consumer marketability, product quality, and health/safety.

Plastics/polymers may be fed with, or without, bran additives; the inclusion of bran additives can increase survival or growth/pupation rates (Lou et al. 2021, Yang et al. 2018) and reduce rates of cannibalism (Yang et al. 2021b). YML can survive and reproduce on some 100% polymer diets (Nukmal et al. 2018) however slow growth rates (Billen et al. 2020), increased metabolic stress (Tsochatzis et al. 2021), and higher mortality rates (Wang et al. 2022a, Jin et al. 2022, Yang et al. 2021a), have been cited as reasons why large-scale rearing on polymers may be impractical. Given trends of higher mortality and cannibalism, feeding polymers to YML (especially without bran additives) would become a welfare concern if producers adopted this practice.

Larval hormonal additives

'Giant mealworms' are used in the exotic pet industry (and thus represent a small percentage of the total farmed YML population). Giant YML are produced by incorporating juvenile hormone (JH), a hormone that controls the post-molt lifestage of the insect, into the larval diet (Finke et al. 2002). If JH titers are high, the post-molt form will be another larva; low titers lead to pupation (Jindra, 2019). Artificial JH in the diet thus ensures YML molt into continuously larger larvae. As a consequence, their continued feeding increases their body size (e.g., 304 mg vs. 126 mg; Finke et al. 2002).

Hormones affecting growth and development have been shown to negatively affect farmed vertebrate welfare through changes in behavioral abilities and physiological states that increase discomfort or pain (Humane Society of the United States, 2016; Scientific Committee on Animal Health and Animal Welfare, 1999). However, there are many growth-related issues in vertebrates that are simply not applicable in invertebrates (e.g., skeletal disorders). In *Drosophila melanogaster* fruit flies with diet-induced obesity, increased fat body deposition and weight correlates with heart dysfunction, hyperglycemia, reduced activity levels, and insulin resistance (Smith et al. 2014). Similarly, giant YML have similar protein and moisture levels to normal YML, but increased fat content and weight (168 vs. 134 mg/kg; Finke et al. 2002). Diet-induced obesity is not a perfect parallel for hormone-induced growth, but these data suggest there could be negative welfare effects resulting from this practice.

Adult nutrition

Adults without food failed to lay eggs and died within 11 days (Dick 1937). Females provided with an excess of wheat bran and potato produced significantly more larvae than those starved for three days prior to being provided with diet, suggesting nutritional quality/quantity can affect reproductive output (Worden and Parker, 2001). Adults in industry conditions are generally reared on a diet of wheat bran with water (and possibly protein) sources, accordingly.

Dietary macronutrient balance (protein: carbohydrate, P:C) is a critical determinant of adult lifespan and reproduction. Average lifespan was the longest for males at P:C 1:1 and longest for females at P:C 1:1 and 1:5, while age-specific egg laying was highest at P:C 1:1. Adults actively regulated their intake towards a slightly carbohydrate-biased ratio (males = P:C 1:1.54–1:1.64; females = P:C 1:1.3–1:1.36; Rho and Lee, 2016). However, components of the diet used for testing adult macronutrient selection were unusual: casein, peptone, and albumen were used for the protein while the carbohydrate 'contained sucrose' (it is unclear if wheat bran or other more typical carbohydrate sources for this species were provided). Indigestible cellulose powder was also the majority of the dietary mixture. Results from this study should thus be interpreted cautiously given that wheat bran is the most commonly reported feed for adults.

Adults may currently be protein-limited if not provided supplemental protein sources (yeast, soy protein, etc.; Ribiero et al. 2018). In general, providing both carbohydrate and protein sources should allow adults to maximize their welfare by managing their own nutrition.

Hydration: Relative humidity (RH) and water

There may be lethal impacts of poor hydration in high temperature environments, especially when individuals are at vulnerable life stages (eggs, adults). High egg mortality was observed at 12% RH (Punzo and Mutchmor, 1978; and see Johnsen et al. 2021); survival was highest at 75% RH (Punzo and Mutchmor, 1980). 12-20% RH reduced adult body moisture and survival (Dick, 1937; Pielou and Gunn, 1940; Punzo and Huff, 1989). Adults not provided with some moisture will die within 4 days, without producing eggs (Dick, 1937).

YML are not as vulnerable to dehydration stress. YML are highly resistant to desiccation-induced mortality and may live for >150 days without water sources relying on metabolic water production, in part due to their ability to absorb moisture from the air through their rectal complex at certain RH (Buxton, 1930, 1932; Machin, 1975; Mellanby, 1958; Mewis and Ulrichs, 2001; Noble-Nesbitt, 1970; Schulz, 1930).

Nevertheless, poor hydration can have lethal or sublethal impacts on fitness/welfare at any life stage. Larval body size and RH are positively correlated, with larvae in high RH (84%) conditions nearly two fold the body mass of those in moderate RH (43%) conditions after three weeks of growth (Johnsen et al. 2021). Larval growth rates are highest at humidities of 85-100% (Hardouin and Mahoux, 2003; Johnsen et al. 2021). YML ceased activity and growth at low RH (e.g. 13-30%; Fraenkel, 1950; Murray, 1968) and pupation rates declined in low RH treatments (Graham et al. 2001). A benefit of the aggregation of conspecific insect larvae is the production of a localized microclimate with higher humidity due to evaporative and excretory water losses (Nicolson, 2008); whether this occurs in YML is unknown. The effects of poor hydration are strain-specific, producing variable effects on development time (Adamaki-Sotiraki et al. 2022), larval weight, and adult longevity at 50% RH (Urs and Hopkins, 1973).

Behavioral evidence suggests that well-hydrated adult YM prefer to live in drier substrates (Harbach and Larsen, 1977), with increasingly strong avoidance at > 70% RH (Pielou and Gunn, 1940). This trend may account for the increase in adult female activity at 90-100% RH (Hardouin and Mahoux, 2003), as females may have sought to escape too-wet conditions. Adults are sensitive to relative humidities as similar as 94% and 100%, with >90% of individuals preferring 94% RH to 100% RH. Individual adults spent 92% of their time on the dry side of a humidity gradient, suggesting dry substrates are strongly preferred, with only brief visits to areas of high moisture (Pielou and Gunn, 1940). Adult RH preferences were also affected by desiccation stress in low RH environments. Intentionally desiccated adults strongly preferred > 90% RH (Harbach and Larsen, 1977).

Supplemental sources of water may be provided *ad libitum* for YM of all life stages though YML may be reared without access to a water source in industry conditions to avoid fungal growth (reported in Adamaki-Sotiraki et al. 2022). Provisioning water sources often requires significant labor for producers as they must be changed regularly to retain the moisture and keep crates clean; to save time, producers may thus place all the ‘wet feed’/‘water’ in the center of the crate instead of distributing it throughout the feed. Producers should always take care to distribute the wet feed throughout the crate, especially for smaller (< 30 mg), less mobile larvae: decreases in growth rate were observed when wet feed was more than 5 cm from the larvae, with resulting larvae up to 150x smaller in body mass when found more than 15 cm from wet feed. This also increased size variability, which may result in product loss during sieving or increase cannibalism (as smaller larvae are more likely to be cannibalized by larger conspecifics; see section ‘Cannibalism’). As a result, Deruytter et al. (2020) recommend distributing wet feed so that it is always within 5 cm of the larvae.

Providing moisture sources allows individual YM to behaviorally regulate their water balance by directly ingesting water (Mellanby and French, 1958; Murray, 1968) or by changing their proximity to the higher RH microclimate near the water source (Barton-Browne, 1964; Ghaly and Alkoik, 2009). YML will move towards water, even when that requires migrating to the surface of a container, suggesting unmet hydration needs might be more stressful than photophobia (Martin et al. 1976). YML have been observed seeking out damp areas in natural and laboratory conditions (Cotton and St. George, 1929; Howard, 1955; Urs and Hopkins, 1973). Providing water sources has been suggested to reduce YML cannibalism (Hardouin and Mahoux, 2003), potentially stopping larvae from seeking water from within conspecifics’ bodies.

Providing water sources increased growth rates and body masses of YML, and increased adult eclosion rates and longevity (at 50% RH, Mellanby and French, 1958; Murray, 1968; Urs and Hopkins, 1973; also, see decreased development times, 70% RH, Oonincx et al. 2015). In addition, water sources may increase larval survival (compare the same strains in Rumbos et al. 2021 with Adamaki-Sotiraki et al. 2022; and see trends in Urs and Hopkins, 1973) and adult survival (Ghaly and Alkoik, 2009). The performance, and probable welfare, benefits of providing supplemental water sources for larvae appear more pronounced at low-to-moderate RHs (Murray, 1968), while water sources are required at all RH for adults.

While moderately high RH may be preferred for welfare, fungi, mites, etc. might proliferate in high-humidity conditions or when water sources are provided (Frankel, 1950; Ghaly and Alkoik, 2009). High RH also increases sensitivity to high temperatures at all life stages (Punzo and Mutchmor, 1978, 1980). In summary, although 85-100% humidity may be best for growth rates at temperatures of 25 °C, 65-75% with supplemental water seems to provide optimal allowance for growth and natural behaviors while reducing the risks of thermal stress and disease that present at higher humidities. In particular, ~65% with a supplementary water source may be preferable for adults (given avoidance data), while ~70-75% with a supplementary water source may be preferable for larvae/eggs.

Environmental pollutants

Some substrates commonly fed to YML may be contaminated with pollutants/pathogens, including entomopathogenic biocontrol agents (see section ‘Microbes’), pesticides, toxins, or heavy metals (van der Fels-Klerx et al. 2018). Other pollutants (e.g., dioxins) accumulate in YML (Poma et al. 2017). However, we did not find any studies on the welfare impacts of those pollutants; additionally, no studies were available on the effects of environmental pollutants on YM adults.

Pesticides/herbicides/fungicides

YMs are frequently provided agricultural products as a food or water sources which may have residual pesticides, herbicides, or fungicides. These chemicals have lethal and sublethal effects in insects (Costa et al. 2008; de França et al. 2017); some can be detected in YML after just one 48-hr exposure (before any significant effects on survival; Houbraken et al. 2016). Direct application of either a pyrethroid, neonicotinoid, or organophosphate to the YML cuticle resulted in increased mortality after 3 days (Maliszewska and Tęgowska, 2016). Contact with a neonicotinoid and a pyrethroid applied to concrete surfaces (0.1 mg/cm^2) increased mortality by 26.7% - 97.8% across all life stages after 7 days. Large larvae were more immediately tolerant of pesticides than small larvae or adults (Athanassiou et al. 2015).

Insect growth regulators (IGRs) have also proven lethal to YML, though some studies only test injection (not relevant for farmed YML). Topical applications of the JH analog pyriproxyfen decreased pupal survival by trapping pupae in their exuvium (e.g., exoskeleton; Aribi et al. 2006). External diflubenzuron application dose-dependently decreased pupal survival, particularly when applied in early pupal stages, and decreased adult longevity (Soltani et al. 1983). IGRs like triflumuron, azadirachtin, and pyriproxyfen also decreased cuticular thickness in YMs (Aribi et al. 2006; Pascual et al. 1990; Soltani et al. 1996). Given the rapid uptake and sensitivity of YM to different pesticides, careful pre-processing of substrates will be required to safeguard welfare.

Plant derivatives (such as glycoalkaloids) are an alternative to synthetic pesticides, and may cause sublethal, negative effects on YML health (Spochacz et al. 2018). However, plant-derived glycoalkaloids are not currently used in large amounts and are unlikely to pose a risk to farmed YML.

Cry toxins

The gram-positive bacterium *Bacillus thuringiensis* produces a family of proteins (Cry toxins) that have taxon-specific toxicity (de Maagd et al. 2001). Cry toxins may rupture the mid-gut epithelial cells of insects, causing rapid death. Alternately, Cry toxins may cause a slow death by 1) causing gut paralysis and starvation (expected to be the most common death resulting from Cry toxins; Graf, 2011), or 2) allowing bacteria to colonize the hemocoel, producing a severe septicemia prior to death (Broderick et al. 2006; Raymond et al. 2010; reviewed in: Hilbeck et al. 2018). Agricultural crops may be genetically modified to produce Cry proteins, or Cry proteins may be applied (this application rapidly degrades in the environment, posing less risk to farmed YM; Van Rie, 2000).

YM are susceptible to Cry3Aa (Fabrick et al. 2009), but not Cry1A, Cry1I, Cry8, or Cry34/35 (Oppert et al. 2011). Time to death is dose-dependent, and can be prolonged: only 25% of YML exposed to 11 mg/kg of Cry3Aa had died by day 30 (compared to 0% of controls); average time to death was 40 days. Absolute mortality (100%) occurred by day 30 when YML were exposed to 42 mg/kg of Cry3Aa, with an average time to death of 18 days. In both cases, insect growth was severely stunted (Oppert et al. 2011). Reduced feeding behavior occurs when YM are infected with *B. thuringiensis* (and not simply exposed to the toxins; Zanchiet et al. 2020) but the specific cause of death for YM (gut rupture, septicemia, gut paralysis, etc.) is currently unknown.

Although Cry proteins may have severe, negative impacts on welfare (via pain, reduced health, etc.), they likely represent a minimal risk to farmed YML. Producers may avoid feeding YML transgenic grains, or those treated with Cry-based insecticides. Additionally, heat of 60 – 90 °C can be used to inactivate Cry toxins (Ujváry, 2010), suggesting appropriate substrate pre-processing could ensure larval safety.

Mycotoxins

Agricultural products (particularly cereals) may be contaminated with mycotoxins produced by fungi/molds. Many mycotoxins have acute or chronic negative effects on the health of vertebrate organisms that consume them (Pier et al. 1980); thus, high mycotoxin levels may cause grain to be destroyed. The IAFF industry is interested in the reclamation of mycotoxin-contaminated grains as feed for YML. Mycotoxin bioaccumulation, and/or the transformation of toxins into more dangerous compounds, can occur in YML (often at low levels; Niermans et al. 2019; Ochoa-Sanabria et al. 2022), representing a potential food/feed safety concern when rearing YML on contaminated substrates (Bordiean et al. 2020; but see Duhra et al. 2021; van Broekhoven et al. 2017).

Mycotoxins may have lethal or sublethal adverse effects on insects depending on toxin, toxin concentration, rearing conditions, species, exposure time, developmental stage, or the combination of mycotoxins produced by a fungal species (Davis et al. 1975; Evans and Shao, 2022; Niermans et al. 2021). One challenge in interpreting potential real-world welfare impacts from studies on mycotoxins is that mycotoxin levels in naturally-occurring contaminated grains can be much higher than those tested. For example, Ochoa-Sanabria et al. (2022) obtained naturally contaminated wheat screenings with an ergot alkaloid (EA) concentration of 787,000 ug/kg; however the highest EA concentration they tested on YML was 15,316 ug/kg (diluted using uncontaminated grains). Similarly, tests of the *Fusarium* spp. mycotoxins, deoxynivalenol (DON) and zearalenone (ZEN), at concentrations up to 12,000 ug/kg yielded no strong negative effects (Table 1 and Table 2). However, naturally infecting feed with *Fusarium culmorum* produced DON levels of >10,000 ug/kg, and ZEN levels of 210,000 ug/kg; this resulted in reduced survival of last-instar YML (Guo et al. 2014). Several mycotoxins have been tested at higher concentrations in other species of Coleoptera. Dose-dependent mortality reached 100% for some species/toxin/concentration combinations (reviewed in Niermans et al. 2021; Fig 2), suggesting that the high concentrations of toxins found in naturally-contaminated substrates may be more toxic to YML than the studies performed using lower levels currently demonstrate.

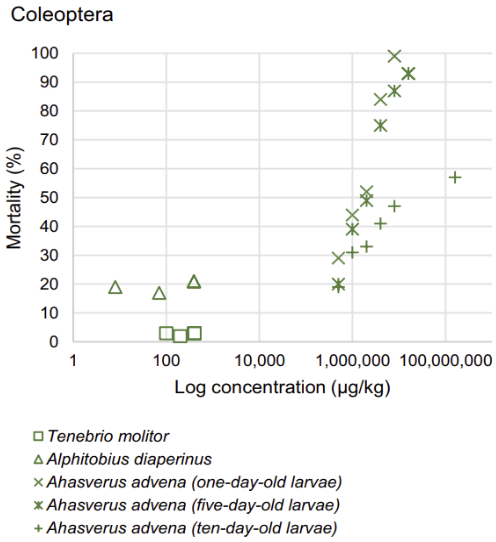

Fig 2 (left). Mortality (%) caused by exposure to different doses of AFB1 (log scale) for Coleoptera. From Niermans et al. 2021 (CC-BY-4.0).

Additionally, many studies looked only at the effect of a single mycotoxin (Table 1), however naturally-occurring fungal contamination typically produces more than one mycotoxin (Table 2), which may synergistically increase toxicity (Canteri de Souza et al. 2018). Studies that look at the effects of mycotoxin mixtures or naturally contaminated grain sources may be a more accurate representation of the welfare impacts of mycotoxin-contaminated substrates on YML. Lastly, many studies are conducted on older YML; however, early-instar larvae are expected to be more susceptible to mycotoxins (Abado-Becognee et al. 1998). These three major methodological concerns mean that the results of many of the mycotoxin studies conducted to date may underestimate the negative welfare effects of mycotoxin-contaminated substrates.

Fusarium spp. mycotoxins such as DON, ZEN, Diacetoxyscirpenol (DAS), fumonisin B1, and HT-2/T-2 toxins did not appear to affect survival in YML (Tables 1 and 2; but see Guo et al. 2014). Aflatoxin B1 (AFB1) produced by *Aspergillus* spp., and EAs produced by *Claviceps* spp., also did not affect survival (Bosch et al. 2017; Ochoa-Sanabria et al. 2022; Zhao et al. 2022). Davis et al. (1975) studied seven species of *Fusarium,* as well as *Pithomyces chartarum, Wallewia* sp. JA 7332, *Gonatobotrys* sp. JA 737-17, *Penicillium viridicatum, Acremonium* sp. MCH 746, *Myrothecium* sp. JA 7319, 7320, MCH 741 & 742, and found survival was generally unaffected. However, a few species of *Fusarium* and *Myrothecium* may decrease survival, especially when temperatures are high (Davis and Smith, 1977). *Fusarium* spp. do not easily colonize YML, suggesting that secondary metabolites are responsible for any reductions in larval survival (Guo et al. 2014).

Sublethal adverse effects were reported for many mycotoxins. Davis et al. (1975), Davis and Smith (1977), and Reiss (1973) reported reductions in growth when larvae were fed substrates contaminated with *Fusarium nivale, F. equiseti,* and *F. roseum, Aspergillus niger* and *A. flavus, Penicillium expansum, Cladosporium herbarum,* and *Myrothecium* spp. (specific mycotoxins unreported). Janković-Tomanić et al. (2019) demonstrate a suite of dose-dependent adverse effects in artificially-contaminated DON diets (4,900 - 25,000 ug/kg), including: decreases in body weight and protein content, increases in antioxidant enzyme activity indicative of oxidative stress, and reduced locomotor behaviors. Fumonisin B1 affects the respiratory metabolism of YML, resulting in reduced growth, feeding behavior, and metabolic rates, and delayed development (Abado-Becognee et al. 1998). Larval growth was reduced by ~50% and 67-78% after four weeks on 128 ppm DAS and T-2 toxin diets; dose-dependent declines in growth were linked to reductions in feed utilization, potentially indicative of behavioral avoidance (Davis and Schiefer 1982, 1983).

Behavioral avoidance of contaminated substrates has been recorded with other mycotoxins: YML reduced their intake of EA-contaminated feed over 21 days, despite no apparent preference/avoidance of contaminated feed after 20 minutes of exposure (Ochoa-Sanabria et al. 2022). Avoidance behavior strongly correlated with toxin-induced mortality (Guo et al. 2014). Behavioral avoidance of fungal contaminants may be the mechanism driving sublethal declines in body size, suggesting malnutrition may be another welfare concern (Howard, 1955).

Overall, these data suggest that many mycotoxins can negatively affect the welfare of YML and, at high concentrations or in specific combinations, these effects can be lethal. If mycotoxin-contaminated substrates cannot be avoided, tests should be performed that ascertain which fungal species and mycotoxins are present, in order to appropriately dilute concentrations where necessary to protect welfare, productivity, and product safety.

In some cases, potential benefits of lower concentrations of mycotoxins for YML were reported: DON concentrations up to 12,000 ug/kg either increased or did not affect body weight (Tables 1 and 2). Increases in body mass were observed following the natural production of mycotoxins, but rarely in artificially spiked diets (e.g., Davis et al. 1975; Reiss, 1973; van Broekhoven et al. 2017). This suggests that increases in weight may be due to other fungal secondary metabolites and not the mycotoxins (van Broekhoven et al. 2017). YML may preferentially feed on grains containing a few specific *Fusarium* spp. for this reason (Guo et al. 2014, 2018). Further research could confirm if certain levels of specific mycotoxins/fungi are actually beneficial to welfare and/or production.

Table 1. Effects of different mycotoxins in artificially contaminated diets on YML welfare.

Toxin	Concent-ration in diet	Larval age	Effects of mycotoxin	Reference
AFB1	13 - 415µg/kg	First instar; reared through pupation	Survival unaffected; slight increase in body mass when fed 204 µg/kg (effect not concentration-dependent), otherwise body mass unaffected	Bosch et al. 2017
AFB1	100 µg/kg	3rd through 8th instars	Survival unaffected; weight gain unaffected for 30 days	Zhao et al. 2022
DAS	0 - 128 ppm	~10 mg in weight; reared for 4 weeks	Survival decreases up to 20%, but not strongly dose-dependent; reduction in growth rate, food utilization	Davis and Schieffer, 1983
DON	8,000 µg/kg	5 weeks; reared 2 weeks	Survival unaffected; body mass unaffected	van Broekhoven et al. 2017
DON	4,900 - 25,000 ug/kg	2 months old; reared through pupation	Survival unaffected; dose-dependent larval body mass and protein reduction; increased antioxidant enzyme activities; reduced locomotion	Janković-Tomanić et al. 2019
Fumonisin B1	50,000 - 450,000 ug/kg	15 weeks old (injection study); 7 weeks old (feeding study), reared through pupation	Survival unaffected in all conditions. When injected, single exposure: acute reduction in metabolic rate, followed by recovery; When fed continuously: decline in growth at 450,000 ug/kg, increased larval instar duration, reduction in diet consumption, reduction in metabolic rate (all around 28 days of feeding)	Abado-Becognee et al. 1998
T-2 toxin	0 - 128 ppm	~10 mg in weight; reared for 4 weeks	Survival unaffected; reduction in growth rate, protein utilization, feeding behaviors	Davis and Schiefer, 1982

T-2 toxin & HT-2 toxin	54 & 52 µg/kg 140 & 120 µg/kg	42 days old; reared 4 weeks	Potential increase in survival on high T2/HT-2 diet**; potential increase in body mass on T2/HT-2 diets	Piacenza et al. 2020
ZEN*	427.0 - 2283 µg/kg	42 days old; reared 4 or 8 weeks	Survival unaffected; change in body mass unaffected	Niermans et al. 2019

*also contained DON: (control: 572 µg/kg) ranging from 568.4 - 2101 µg/kg; other mycotoxins may be present and not analyzed

**statistics not provided for comparing artificial-control to artificial-contaminated diets

Table 2. Effects of different mycotoxins in naturally contaminated diets on YML welfare.

Fungal species	Main toxins*	Concentration in diet	Larval age	Effects of mycotoxin	Reference
Not listed	BEA DON** EAs ENA1 ENB ENB1 NIV ZEN	13 µg/kg 4,900 µg/kg 21 µg/kg 45 µg/kg 490 µg/kg 170 µg/kg 270 µg/kg 73 µg/kg	5 weeks; reared 2 weeks	Survival unaffected; increased body mass	van Broekhoven et al. 2017
Not listed	DON ZEN	2854 or 4588 µg/kg 602.3 or 919.3 µg/kg	42 days old; reared 4 or 8 weeks	Survival unaffected; increased growth rate compared to control and artificial diets	Niermans et al. 2019
Not listed	EAs	(control: 63 µg/kg) 3,863 - 15,316 µg/kg	7th-9th instar; reared 21 days	Survival unaffected; growth rate unaffected; reduced feed intake; no preference/avoidance 20 minutes after initial exposure	Ochoa-Sanabria et al. 2022
Not listed; ‘ergot’	Not assessed	1% - 20%	~10 mg larvae; reared for four weeks	Survival unaffected; growth rate reduced in 20% compared to control	Davis et al. 1975

F. culmorum	DON ZEN	10,240 µg/kg >210,000 µg/kg	Last instar, reared 15 days	Reduced survival; increased body mass; behavioral preference	Guo et al. 2014
F. avenaceum	ENA ENA1 ENB ENB1	14,260 µg/kg 60,510 µg/kg >90,000 µg/kg >90,000 µg/kg	Last instar, reared 15 days	Reduced survival; reduced body mass; behavioral avoidance	Guo et al. 2014
F. poae	BEA ENB ENB1	30,100 µg/kg 320 µg/kg 30 µg/kg	Last instar, reared 15 days	Survival unaffected; increased body mass; strong behavioral preference	Guo et al. 2014
F. proliferatum	BEA ENA ENB ENB1 FB1	36,360 µg/kg 30 µg/kg 27,090 µg/kg 2,890 µg/kg 39,740 µg/kg	Last instar, reared 15 days	Survival unaffected; increased body mass; strong behavioral preference	Guo et al. 2014
F. graminearum	DON‡	(control: 200 µg/kg) 2,000 - 12,000 µg/kg	Whole life cycle	Survival to pupation unaffected, pupal survival reduced; body mass of larvae unaffected; reduction in adult body weight of the second generation; no preference/avoidance	Ochoa-Sanabria et al. 2019
F. sporotrichioides	T-2 toxin HT-2 toxin	11 & 89 µg/kg 26 & 262 µg/kg	42 days old; reared 4 weeks	Survival unaffected; increased body mass	Piacenza et al. 2020

*Abbreviations: Beauvericin (BEA), Deoxynivalenol (DON), Ergot Alkaloids (all types; EA), Enniatins (EN), Fumonisin B1 (FB1), Nivalenol (NIV), Zearalenone (ZEA). **In all cases, other toxic products may be present and were simply not analyzed.**

**modified DON present: Deoxynivalenol-3-glucoside - 300 µg/kg; 15-acetyldeoxynivalenol - 86 µg/kg

‡modified DON present: 3-aceytldeoxynivalenol - <LOD, 63, 52, 205 µg/kg per diet.

Heavy metals

Insects that ingest heavy-metal-contaminated agricultural products, soil, or other substrates will accumulate these metals, such as lead, arsenic, and cadmium, in their tissues (Bednarska and Świątek, 2016; Cappelli et al. 2020; Mlček et al. 2017; Poma et al. 2017; van der Fels-Klerx et al. 2016; Vijver et al. 2003). Bioaccumulation can depend on the metal, its concentration, and the life stage of the insect (van der Fels-Klerx et al. 2018). The bioaccumulation of As and Cd in YML tissues may sometimes exceed levels considered safe for animal feed or human consumption (Mlček et al. 2017, van der Fels-Klerx et al. 2016; but see: Bordiean et al. 2020, Poma et al. 2017). Effects on YML welfare are untested.

Heavy metals change the gut microbiome composition of other insect species, and may increase pathogenic bacteria populations that negatively impact host health (Li et al. 2021; Wu et al. 2020b; no studies have tested the effects of heavy metals on YM microbiomes). They may also affect insect weight gain and survival, though this is often context-dependent (e.g., Biancarosa et al. 2017 vs. Purshke et al. 2017). YML survival decreased from 80.7% in the control to ~40-65% in an array of substrates containing increasing As, Pb, and Cd concentrations. Body mass also declined from 4.7 (control) to 2.0-3.6 g (heavy metals) over the same developmental period. However, many of these differences (particularly, in survival) were not statistically significant or strongly concentration-dependent, and may also have been caused by the acid used to mix metals into the food (van der Fels-Klerx et al. 2016). It is thus challenging to interpret the welfare impacts of heavy metals on YML from these results.

Abiotic conditions

Abiotic conditions, nutrition, and interspecific interactions may synergistically or antagonistically affect an individual's welfare. The strength and direction of these interactive effects may depend on life stage (e.g., see section 'Hydration'), or population (Adamaki-Sotiraki et al. 2022). Scale is known to impact many aspects of insect survival, reproduction, etc., in a non-linear fashion (Park, 1933; Scala et al. 2020; Sørensen et al. 2012; Yang and Tomberlin, 2020). Producers should thus exercise their best judgment when applying recommendations for abiotic conditions to their populations in order to maximize welfare.

Temperature (rearing)

Insects are poikilotherms (e.g., largely ectothermic); ambient temperature is critical to maintaining an optimal internal body temperature (Huey and Stevenson, 1979). Insects may still survive at a range of non-optimal temperatures. However, these temperatures may negatively affect their development, physiology, or behavior sublethally (Du et al. 2007; Harvey et al. 2020).

Ribiero et al. (2018) reviewed the impacts of temperature on YM; we briefly review their conclusions and incorporate more recent literature. The best temperatures for rearing YM appears to be 25 - 28 °C, irrespective of humidity (Eberle et al. 2022; Parsa et al. 2023; Ribiero et al. 2018), though growth rate was maximized with higher mortality at 31°C (Bjørge et al. 2018; but see increased development times at 30 °C v. 25 °C in Ludwig, 1956). Temperatures greater than 35 °C result in reduced survival across life stages. Low temperatures (e.g., less than 17 °C) adversely impact egg development (Koo et al. 2013) and slow larval development (Lengerken 1925). Temperature by itself does not appear to affect survival differently across life stages (Ribiero et al. 2018); however, the interaction between temperature and relative humidity can affect early- and late-stage larvae, pupae and adults, differently (Punzo and Mutchmor, 1980). Interactions between temperature and photoperiod can also affect development and survival (Eberle et al. 2022). Producers should consider the matrix of these effects in a life stage-dependent manner for their population.

YM critical thermal maxima are life-stage and humidity dependent, and reported to be around 40–44 °C (Ribiero et al. 2018; Stevens et al. 2010). However, in more rapid assays, 46–

47 °C was the CTmax for larvae and pupae (48 °C for adults; Vorhees and Bradley, 2012). YML often aggregate within their feeding substrate; this may increase the local temperature via metabolic heat production by > 10 °C for late-stage larvae (Michal, 1931a; Morales-Ramos and Rojas, 2015). When combined with the insulating effect of the substrate, these localized hot spots could be lethal at high densities (e.g., an in-crate temperature of 43.9 °C was reported in Deruytter et al. 2022). Changing substrate textures and crate materials may allow for more rapid heat dissipation, protecting larvae from overheating.

Overall, rapid increases in temperature, such as localized hot spots, are bad for survival and welfare. However, the prevalence of mortality from overheating is unknown (but see sublethal effects, Morales-Ramos and Rojas, 2015). Reducing thermal variability may be good for both productivity and welfare, due to the physiological costs imposed by large fluctuations in environmental temperature (Arias et al. 2011). However, a small amount of thermal fluctuation may provide hormetic benefits (Colinet et al. 2015).

Temperature (cold storage/slaughter)

YML or pupae may sometimes be stored refrigerated when shipped to pet stores or direct to consumers, in order to extend the duration of time spent at that life stage. 98% or more of 2–3 month old larvae survived 120 days at 10 °C (63% RH). However, 13–19% of larvae did not survive to adulthood, compared to 1.5% of controls (Errico et al. 2021). 30 mg may be the critical weight for cold storage of larvae at 10 °C, as smaller larvae saw increased mortality (Punzo and Mutchmor, 1980). Reduced survival after 10 °C storage suggests being reared in these conditions for a prolonged period results in poor welfare, even for older larvae.

Cold storage of pupae at 4 °C for 15 days increased mortality (75%) and adult deformity (nearly 70%), compared to only 1 day (Sömnez and Koç, 2019). Similarly, increasing the amount of time larvae were in cold storage at 4 °C increased mortality and adult deformation rates, with especially dramatic effects for young larvae (Sömnez, 2021). As prolonged cold storage at 4 °C is frequently fatal, or results in behavior-relevant morphological deformities, it is not recommended for YM welfare.

Freezing in air may also be used as a method of slaughter. It’s important to note that a species’ physiology could affect the time-to-death, and humaneness, of this slaughter method. For instance, YML are freeze-avoidant and frequently overwinter as larvae, thus having lower spontaneous freezing temperatures than adults (Constantinou and Cloudsley-Thompson, 1985). To survive these conditions, YML fat bodies produce small Cys- and Thr-rich thermal hysteresis ‘antifreeze’ proteins that lower the freezing point (supercooling point; SCP) of their hemolymph (Patterson and Duman, 1978). These proteins interact with ice crystals in order to halt ice formation and thus prevent damage to the surrounding tissue (Duman, 2001). Exposure to cold, starvation, and desiccation also increase levels of thermal hysteresis proteins comparably, over a period of weeks. Ultimately, these proteins increase desiccation resistance and lower the SCP (Patterson and Duman, 1978).

The SCP was reported to be between -10.3 and -11.1 °C for YML (no instar noted; Constantinou and Cloudsley-Thompson, 1985), though SCPs were found to be as variable as -1.5 to – -24.3 °C, dependent on body mass/developmental stage (mean = -12.4 °C). -24.3 °C is lower than typical freezer temperatures (-18 °C) and thus some YML may plausibly survive through freezing into the next processing phase (Johnston and Lee, 1990; though, larvae may die before their SCP due to other chill-induced injuries, Andreadis and Athanassiou, 2017). Slaughter age larvae produce more thermal hysteresis proteins than younger larvae (Patterson and Duman, 1978), but the earliest instars have the lowest SCPs (suggesting other factors beyond thermal hysteresis protein levels may matter for determining larval SCP). If larvae are slaughtered at the recommended 100 mg, their mean SCP will be around -14.4 ℃ (Johnston and Lee, 1990).

Importantly, chill coma (the temperature at which the nervous system of the insect is presumably inactivated, and therefore pain can no longer be experienced) occurs long before the SCP. YML chill coma temperatures are reported between 0.5 and 1.3 ℃ at the fifth instar (Arias et al. 2011). Cooling from room temperature to 0 ℃ occurs much more rapidly than cooling beyond 0 ℃ for YML at -20 ℃; it can take 3–12 minutes for individually-frozen YML to reach their SCP and 2 minutes for adults (which have an SCP of -7.1 ℃; Constantinou and Cloudsley-Thompson, 1985). Because large numbers of aggregating insects produce metabolic heat, cooling times to the point of chill coma or the SCP will invariably take much longer for mass-frozen individuals. Altogether these data on YML physiology demonstrate that slaughter by freezing in air in typical -18 ℃ freezers may take a particularly long time for mass-reared YML because of their low SCPs, especially when SOPs include rearing on dry substrates and/or fasting prior to slaughter (as dehydration and starvation increase thermal hysteresis protein levels). The welfare impacts of non-instantaneous cooling, at any rate, is not well understood for insects.

Atmospheric Gas Levels

Environmental oxygen levels are typically around 21% (normoxia). Hypoxia (10%) reduced growth rates, increased mortality, and caused developmental abnormalities in YML, including the inability to develop pigment which may increase susceptibility to disease (Clark and Cristofalo, 1961; Greenberg and Ar, 1996; Loudon, 1988). Hypoxia was significantly worse for survival than normoxia or hyperoxia (40%), with 20%, 96%, and 96% survival to pupation, respectively. Hyperoxia reduced the number of YML molts (no effect on growth rates or final body size; Greenberg and Ar, 1996).

Mass-reared insects produce carbon doxiode (CO_2), ammonia (NH_3), methane (CH_4), and nitrous oxide (N_2O) gasses, though ammonia levels appear to be particularly low in *T. molitor* (though insect gas emissions are partially dependent on feed characteristics, e.g., Lindberg et al. 2022). Active, growing YML fed *ad libitum* are likely to produce the highest gas emissions (compared to adults, or inactive/unfed individuals; Oonincx et al. 2010). The risk of hypoxia in poorly ventilated rearing containers is thus highest during ‘night’ conditions, when YML oxygen consumption and gas production are highest due to their increased activity (Michal, 1931b; Oonincx et al. 2010). High levels of carbon dioxide (CO_2) can also cause insect spiracles to remain open, increasing water loss (Nicolas and Sillans, 1989); desiccation stress is known to slow YML growth (see section ‘Hydration’).

Though hypoxia seems unlikely to affect farmed YMs (except, perhaps, during transport), producers should ensure good air flow in crates; closed systems can accumulate gasses quickly. A closed YML rearing system caused a 2.5x increase in average CO_2 levels, to 1000 ppm (with fluctuations in CO_2 concentrations from 1.25 to 15x that of the open environment); increased CO_2 correlated with reduced larval growth (Li et al. 2015). Other high-density studies under farm-relevant conditions showed CO_2 levels reaching 1500 ppm (Deruytter and Coudron, 2019). Providing appropriate light cycling (see section ‘Light’) will encourage periods of decreased and increased activity, respectively. Reductions in activity reduce gas output, providing time for hypoxic conditions to be cleared out prior to the next ‘night’ period, eliminating extreme and potentially stressful fluctuations in oxygen levels (e.g., Li et al. 2015).

The effects of hyperoxia on economic productivity and animal welfare could be further explored. Hyperoxia may increase insect body sizes and fecundity over multiple generations. Negatively, hyperoxia may also increase oxidative stress (Harrison et al. 2010); adult female YM prefer to mate with males not experiencing oxidative stress (Ruiz-Guzmán et al. 2020).

Light

YM adults are nocturnal and photophobic (Balfour and Carmichael 1928; Cloudsley-Thompson, 1953; Sheiman and Kreschenko 2010). Larvae also demonstrate a strong preference for darkness (Howard, 1955; Loeb, 1905); YML bury themselves in the feeding substrate when exposed to light and will move away from introduced light even when no substrate is provided (Balfour and Carmichael, 1928; Howard, 1955). In fact, both larvae and adults avoid light more strongly than the lethal insect repellant, paradichlorobenzene (Howard, 1955). Light may be necessary during rearing so that facility staff can safely complete their work. YM have low spectral sensitivity to red, like most insects, and thus behave as if they are in the dark when maintained under red light conditions (Loeb, 1905; Wang et al., 2022b, Yinon, 1970). Therefore, using only red light (600-700 nm) may allow personnel to see while reducing light-associated stress for the insects.

Photoperiods for rearing YM populations have included: 18:6, 16:8, 14:10, 12:12, 10:14, and 0:24 light-dark (reviewed in Eberle et al. 2022; Ribiero et al. 2018). Activity increases during the ‘night’ part of the cycle for YML (Oocincx et al. 2010); providing periods of light and darkness may thus allow for natural periods of high and low activity to be expressed. YM behaviors and the visual system are regulated by a circadian rhythm even when maintained at 0:24 or 24:0 (Erber, 1975; Fondacaro and Butz, 1970; Ro and Nilsson, 1993; Wada and Schneider, 1968; though see Cloudsley-Thompson, 1953; Howard, 1955). Larval development may occur more quickly in 14:10 L:D conditions (but see Eberle et al. 2022), alongside increased eclosion rates (Kim et al. 2015; though other studies show no effect: Zim et al. 2022). Photoperiodic responses are affected by other environmental variables, making the generalizability of these patterns unclear (e.g., Kim et al. 2015 vs. Tyshchenko and Ba, 1986).

Although some data tentatively suggest larval development may occur more quickly in long-day conditions, this does not necessarily mean these conditions are good for YML welfare. For example, increased day length when rearing chickens can result in faster growth but causes a host of physiological welfare concerns (Schwean-Lardner and Classen, 2010). Data from chickens are not likely to be directly relevant to YM; however, the example demonstrates why caution should be employed when trying to draw conclusions about welfare using growth rate as a single metric. Data on mortality, behavior, stress hormones, or morphological abnormalities could assist in generating a more accurate picture of YM welfare under different photoperiods. In addition, the effects of photoperiod on adult mating, oviposition, feeding, and locomotor behaviors have not been studied.

Stocking/Rearing Density

Larvae

Surprisingly few studies have directly assessed the impact of rearing density (areal or volumetric) on larval growth parameters, survival, or behavior under standardized abiotic conditions (Table 3). Increased density appears to decrease total weight gain, food consumption (particularly for later-instar larvae), and survival (Deruytter et al. 2022; Morales-Ramos and

Rojas, 2015; though see Deruytter and Coudron, 2019. It's true that YML grow *faster* at ~0.33 than ~0.03 larvae/cm^2, but they reach a smaller pupal weight; Weaver and McFarlane, 1990). Optimal densities are not well-established, but may be lower than the rearing densities that are possible to achieve in farmed conditions.

When comparing the welfare of insects to vertebrate livestock, it is frequently suggested that rearing insects at high densities in intensive production systems is not likely to be detrimental to their welfare because insects are often found in nature at high densities (e.g., Dossey et al. 2021; Erens et al. 2012; IPIFF, 2019; and see discussion of this issue in Delvendahl et al. 2022). First, it should be noted that natural conditions do not necessarily serve as a guide to high animal welfare. For example, just because insect diseases are common in the wild, does not imply that farmed insects should be given diseases to improve their welfare. Similarly, high densities in natural conditions may simply result from overutilization of scarce resources, not because insects' welfare is better under those conditions.

Second, it should be noted that almost no studies have documented the density of insects in natural conditions nor the prevalence of these densities in natural conditions (where we may also expect a bias towards the documentation of larger/high density populations that are easier to detect, as discussed in studies of other group-living animals: Markham et al. 2015; Sharman and Dunbar, 1982). It is thus unclear what quantifiable density insect producers should mimic, even if mimicking natural conditions was expected to produce the best outcome for welfare.

Finally, studies that robustly test the hypothesis that high density conditions improve, or harm, wild insect welfare are non-existent. However, high density conditions can substantially increase mortality due to density-dependent factors (e.g., risks of some diseases, resource scarcity, etc.; Lack, 1954). Indeed, resource scarcity can even increase clutch sizes in some insects (Pilson and Rausher, 1988; Heard, 1998) - an evolutionary strategy to outcompete conspecifics via the sheer number of offspring produced - which may drive particularly low-welfare conditions for most members of the population, alongside very high mortality rates. It is important to be critical of the claim that insect welfare is improved under high density natural conditions, and thus that these conditions should be mimicked in production facilities, until further research on species-specific welfare at particular densities has been conducted.

Nevertheless, and just for the sake of argument, let's suppose that YML thrive under higher density conditions. Still, it remains the case that stocking densities in intensive production facilities could still easily exceed their optimal 'high' density (Table 3). For example, Deruytter et al. (2022) found that the highest tested larval density in crates (8 larvae/cm^3) resulted in substantial reductions in survival (67% survival at 4 weeks, compared to >95% at 9 weeks in other treatments). Indeed, optimal growth conditions for larvae were 0.5 larvae/cm^3 - which was the lowest density tested, suggesting that optimal larval density for individual growth and welfare could be even lower. Granted, rearing larvae at this density resulted in low total yield, increased space utilization, and feed waste. The authors thus recommend 1 larvae/cm^3 to try to minimize the economic and ecological costs of low density production. Similarly, Deruytter and Coundron (2019) recommend rearing larvae at higher densities that resulted in increased mortality (likely due to nutritional resource competition) in order to optimize yield. These studies suggest that there is a tension between maximizing yield and animal welfare, where the conditions that are optimal for the former—including higher stocking densities—are less than optimal for the latter.

Alongside resource competition, physical contact may mediate the negative effects of too-high larval density through the continued internal production of juvenile hormone. YM pupation is delayed through both vibrations and physical contact (Tschinkel and Willson, 1971). Further, consuming large amounts of conspecific frass, which contains compounds with juvenile hormone activity (Schmialek, 1961), may delay pupation (Weaver and McFarlane, 1990). Frass removal may occur using mesh-bottomed rearing containers in industrial systems (Cortes Ortiz et al. 2016); alongside the common practice of pre-pupation slaughter, delays in pupation may be uncommon in farmed settings. However, frass removal (or increased handling, which were confounded) may decrease survival in young YML (Deruytter and Coudron, 2019). Cannibalism and wandering behaviors are also increased at higher densities (Weaver and McFarlane, 1990) which could be behavioral indicators of stress.

It is important to consider that larvae do not distribute themselves randomly throughout a feeding substrate, but instead tend to aggregate (as mediated by chemical signaling: Weaver and McFarlane, 1989; Weaver et al. 1989, 1990). Therefore, absolute larval numbers/mass (and absolute crate size) may matter - not just density per unit area. Additionally, larval density can be measured in the number of individuals or the biomass per unit area. However, individuals of larger mass will be more crowded than the same number of smaller individuals (e.g., earlier instars) in a space, likely leading to more physical contact. Thus, measuring density via body size and not simply number of individuals may more accurately represent the stressors to the larvae.

Table 3. Effects of different volumetric or areal YML stocking densities under controlled abiotic conditions.

Density	Age	Abiotic conditions	Effects	Reference
1 - 20/ 400 ml*	Reared from 1 day old through adulthood	30 °C 55% RH 14:10 L:D Water provided (soaked cotton) Diet: wheat bran and whole wheat flour Food/larvae differed in treatments (400 ml food provided at all densities)	Increased body mass and survival unchanged with increased density at 1 month; female adults have higher body mass in low density trials; survival to pupation and adulthood declines with density	Weaver and McFarlane, 1990
44-351/dm^2	4th-6th instar larvae, reared for 12 weeks	27 °C 75% RH 0:24 L:D No water provided Diet: wheat bran (80%), dry potato, dry egg whites, and soy protein (20%) 34 mg food/larvae	Weight gain and food consumed decreased with increased density; no effects noted on survival or cannibalism	Morales-Ramos and Rojas, 2015
7.38 or 14/ cm^2	12th-13th instar larvae, reared for 27 days	25 °C 70-80% RH 12:12 L:D Water provided (fresh ivy gourd leaves)	No difference in growth rate; 100% survival and no cannibalism in either condition	Zaelor and Kitthawee, 2018

		Diet: wheat bran Feed given at 60% larval weight *Larvae were fasted every 3 days, for 3 days*		
100 - 700/ 2064 cm^3 (e.g., 0.05 - 0.34/cm^3)	40 days after hatching, reared until pupation	25 ℃ 65% RH No photoperiod data Water provided (fresh fruits and vegetables, water sprayed on towel cover) Diet: wheat bran, ‘livestock feed’ Food/larvae differed in treatments (300 g food provided at all densities)	No effect of larval density on pupal weight	Park et al. 2012
0.5 - 8/cm^3, with areal varied from 0.5 - 64/ cm^2	3-4 week old larvae, reared for 9 weeks[↑]	27 ℃ 60% RH 0:24 L:D Water provided (‘wet feed’ agar gel) Diet: wheat bran Food/larvae differed in treatments (1-8 cm food provided to generate different volumetric densities)	Highest density (8 larvae/cm^3) reduced survival (68% at 4 weeks, compared to >95% at 9 weeks in other treatments); lowest densities increased growth rate and final larval weight	Deruytter et al. 2022
Area varied from 0.6 - 10.4/cm^2, with volumetric stable at 1/cm^3	3-4 week old larvae, reared for 6-9 weeks	27 ℃ 60% RH 0:24 L:D Water provided (‘wet feed’ agar gel) Diet: wheat bran Food/larvae same in treatments (1 cm^3 food provided per larvae)	In conditions where frass was not removed, and substrate added, weekly: decreased survival with density; weight increased, then decreased, with density In conditions where frass was removed, and substrate added, weekly: no change in survival with increased density; increased weight with increased density	Deruytter and Coudron, 2019
1-16/78.5 cm^2	age-matched, no data	No temperature data No RH data No photoperiod data	Delayed pupation with increased density	Tschinkel and Willson,

	provided	Water provided (damp filter paper) Diet: wheat bran Food/larvae differed in treatments (30 g food provided at all densities)		1971

*the study says larvae were reared in 455 ml jars, and thus quotes density as individuals/455 ml. However, larvae are unlikely to float in the 55 ml of air above the 400 ml of provided substrate. So, here we provide the density as the # of larvae/unit substrate, which was 400 ml.

†Abstract lists incorrect densities; see Table 1 of study for correct densities.

↑Deruytter et al. 2022 write "the high-density crates (8 larvae/cm^3) were harvested after 4 weeks, as larvae health issues prohibited further continuation."

Adults

Varying adult density affects reproductive output. Zim et al. (2022) found egg production/female declined from 120 to 40 eggs when density increased from 0.25 to 1.5 adults/cm^2 (0.11-0.21 beetles/cm^2 was also optimal for larvae/female/day, compared to 0.42-0.84 beetles/cm^2; Berggreen et al. 2018). Similarly, in Morales-Ramos et al. (2012) the highest reproductive output per female was observed at the lowest densities of 1.4–2.8 adults/100 mm^2 (1:1 sex ratio), while densities of 23.8–84 adults/100 mm^2 resulted in 3 to >10 fold reductions in eggs/female.

This is partially a result of increases in egg cannibalism at higher densities, as separating eggs and adults using steel mesh reduced but did not eliminate the negative effect of density on reproductive output (Deruytter et al. 2019). Deruytter et al. posit that the accumulation of defensive secretions that reduce substrate quality may also contribute to the negative effect of density on reproductive output. If this hypothesis bears out, the stressors that cause the defensive compounds to be secreted could also be a contributor to low reproductive output and welfare. Density may also result in reduced oviposition rates and fitness, as in other Tenebrionid beetles (Longstaff, 1995).

Increases in egg cannibalism at high densities may suggest something about the rearing environment is inadequate for adults in those conditions, perhaps in relation to nutrition or hydration. Alternatively, this may simply be an artifact of females encountering non-kin eggs more frequently, where consumption of non-kin eggs is adaptively beneficial at high densities in order to reduce conspecific competition faced by their own offspring (e.g., Weaver and McFarlane, 1990). Further research is thus needed to confirm if egg consumption should be considered an indicator of negative welfare states for adults. Using steel mesh to separate adults from the underlying oviposition substrate prevents adults from engaging in natural behaviors (namely, burying themselves within the substrate) and may expose photophobic adults to more light (and potentially other stressors), presenting a welfare concern. Similarly, the use of diatomaceous earth to inhibit egg cannibalism (e.g., Shostak, 2014) may result in negative welfare consequences if 1) abrasions are severe enough to reach nociceptive sensory cells underlying the cuticle, 2) dehydration occurs, as the cuticular hydrocarbons that reduce desiccation stress are eroded; or 3) levels are high enough to induce mortality in some beetles.

Crowding is also known to affect female pheromone production. In general, it has been expected that these effects would benefit males in locating receptive, unmated females (Happ and Wheeler, 1969). However, crowding in production environments may exceed levels generally found in natural populations, where enhanced female pheromone production in crowded conditions is expected to be adaptive. Therefore, production-level densities could have unstudied, but detrimental, impacts on YM reproduction via effects on female pheromone production.

Finally, colony-level and individual reproduction may not be maximized under the same rearing densities; while 1.4–2.8 adults/100 mm^2 increased individual-level output, colony-level output was maximized at 8.4 adults/100 mm^2 (Morales-Ramos et al. 2012). Zim et al. (2022) found total reproductive output/box was highest at their highest tested density: 1.5 adults/cm^2. Deruytter et al. (2019) found no optimum output was reached, even at 231.8 adults/100 mm^2. Given that adults aggregate for mating (i.e., do not follow a random pattern of dispersal in the substrate), producers should carefully consider whether density recommendations are affected by the total number of individuals in a crate (though see Deruytter et al. 2019). More studies are needed on the interaction between abiotic factors, substrate quality, crate size, and adult rearing density.

Physical Health and Disease

YM welfare may be impacted by interspecific interactions. Many of the studies that examine how interspecific interactions affect YM growth or survival occur at laboratory (e.g., benchtop) scales. However, scale (and density) may be particularly important when considering the welfare impacts of pathogens or parasites, due to their effects on transmission dynamics, disease progression, etc. This, alongside a paucity of data on the pathogens and parasites most common in the industry, may limit the applicability of some of our conclusions to industrial conditions. Here, we review interspecific interactions (predators, pathogens, and parasites) that may affect YM welfare in farmed conditions.

Predators

In their natural habitat, YM are attacked more frequently by invertebrate than vertebrate predators (Nimalrathna et al. 2023). A variety of arthropods will accept YM as prey including stink bugs (Neves et al. 2010; Zanuncio et al. 2011), assassin bugs (Yuliadhi et al. 2015), jumping spiders (Strohmeyer et al. 1998), wasps (Barr et al. 1996), and mantids (Mirzaee et al. 2022). Because of their limited mobility, larvae and especially pupae may be most vulnerable to predation. Vertebrate grain pests will opportunistically consume YM.

The most relevant predation context for farmed YM will be those sold directly to consumers to be used as live feed for exotic pets (e.g., reptiles), birds (chickens), mammals, and fish (indicating all could act as predators in a farmed context as well). The welfare concerns of predation for YM are not well understood, but may include: body damage, potential fear or distress from the presence or actions of predators (as indicated by behavioral changes or avoidance in other insects; Cinel et al. 2020), or mortality. Predation by larger vertebrates may be better for YM welfare if they can be swallowed whole, quickly, and without injury (rarely the case for smaller invertebrate predators, or small vertebrates like bats).

Microbes: Viruses, bacteria, protists, and fungi

YML are an important insect model for humoral and cellular aspects of innate immunity (e.g., Jo et al. 2019; Johnson et al. 2014; Petronio et al. 2022; Vigneron et al. 2019). Immune responses to a wide range of parasites and pathogens have been characterized (Haine et al. 2008;

Jo et al. 2021). This information may be useful to producers if practices can facilitate behaviors, cellular responses, or molecular pathways identified in preventing or overcoming pathogens.

The first barriers to pathogenic microbes are behavioral. Insects may directly reduce exposure through avoidance (qualitative resistance), reduce infection through clearance (quantitative resistance), or shift reproductive investment to compensate for decreased health or longevity (tolerance; de Roode and Lefèvre, 2012). For example, YML avoid grains colonized by the insect pathogenic fungi, *Beauverina bassiana* and *F. avenaceum*, but appear to show preference for grain infected with other *Fusarium* species (see section 'Mycotoxins'; Guo et al. 2014). Another example is larval avoidance of rodent feces containing the tapeworm *H. diminuta* (Shea, 2010). Housing or food delivery structures that allow freer movement may facilitate these innate, beneficial behaviors.

The next barriers to disease are physiological. Microbes enter insects through feed consumption, tracheal or genital openings, or by breaching the cuticle and underlying epidermis. In addition to being a physical barrier, the layered cuticle increases antimicrobial activity when exposed to bacteria following abrasion (Brey et al. 1993). When large, foreign particles such as parasitoid eggs or fungal conidia reach the hemolymph, the insect immune system mobilizes a fast, constitutive response of the prophenoloxidase (PO) cascade, engulfing and melanizing the particles. Upon bacterial invasion, innate immune response of germline-encoded pathogen recognition receptors detect the microbes, instigating an immune response that culminates in the upregulation of antimicrobial peptides (AMPs; reviewed in Siva-Jothy et al. 2005; Keshavarz et al. 2020). AMPs help maintain homeostasis in the intestinal flora by suppressing overgrowth or spread of particular endosymbionts (Login et al. 2011).

Genetics also plays a role in immune function. Cuticular melanin is produced as part of the phenoloxidase (PO) cascade, playing a role in the encapsulation response. In YM, heritable variation in cuticle color correlates with immune responses: dark-colored beetles, with higher constitutitive PO activity, had more robust responses to immune challenge and were more resistant than light-colored beetles to the fungus *Metarhizium anisopliae* (Armitage and Siva-Jothy, 2005; Barnes and Siva-Jothy, 2000). There may be a tradeoff to consistently elevated PO activity as it can result in increased, system-wide oxidative stress, though darker individuals do not appear to suffer decreased fecundity or lifespan (Krams et al. 2016).

The vast majority of described interspecific interactions are detrimental to insects, and therefore can be expected to have negative impacts on their welfare. Endoparasites, for example, may influence an insect's welfare by changing their behavior (e.g., movement, feeding, or memory deficits; described throughout), inducing malnourishment, damaging or distending the body (Eilenberg et al. 2018), or even causing mortality. YM are host to a number of endoparasites including viruses, bacteria, protists, and fungi (microsporidian infection is rare, and so we do not cover it here; but see: Slowik et al. 2023). Most of these enter through the alimentary tract, but a few can also enter opportunistically through breaks in the cuticle; some fungi actively breach the cuticle, growing through it to gain entry. Virulence can range from nearly undetectable to rapid symptom onset and death.

Both the severity and duration of welfare impacts, prior to death, will be relevant to the insect's welfare. Although not yet validated as welfare indicators for insects, reductions in growth or reproductive output may also suggest welfare concerns when a disease-causing agent is present. The sublethal welfare impacts of most parasites and pathogens have not been studied; we thus are most likely to underestimate the welfare concerns they present without additional research. To best understand the welfare of farmed insects it is important to consider both detrimental and beneficial relationships (e.g., YML glean benefits from microbiota; Lecocq et al. 2021). However, here we will largely focus on pathogens and parasites that may reduce welfare through incidence of symptomatic disease or painful death. Most of our knowledge of intraspecific interactions centers on the larval stage, but we have noted effects on adults, where studied; it is unclear if research attention bias or reduced incidences of disease underlies the difference in literature on adults vs. larvae.

Viruses

Two viral taxa have been identified as potentially infecting *T. molitor*: insect-specific parvoviruses (Family Parvoviridae, genus *Densovirus*) and Iridescent viruses (IIVs; Family Iridoviridae, genus *Iridovirus*; Bertola and Mutinelli, 2021).

It is possible that YML may serve as vectors for *Acheta domesticus* densovirus (AdDNV): AdDNV was isolated from YML mass-reared alongside AdDNV-positive *A. domesticus* crickets (Szelei et al., 2011). However, viral particles were prevalent throughout the rearing facility (e.g., in air filters) and the authors did not verify if the viral particles were simply present on the external surface of the YML or if there was actually internal infection. YM were tested as a potential model for studying crustacean densovirus (PmergDNV), but injection with this virus resulted in only very low levels of viral replication (La Fauce and Owens, 2008). Further, densoviruses have not been reported in natural populations (though these populations are undersampled). There is, therefore, little evidence to implicate densoviruses as significant agents of disease in farmed YM without further research.

Insect iridoviruses (IIVs) are a more common and widespread agent of disease in YM. They are named for the characteristic iridescent hue of infected larvae or pupae, caused by crystalline structures of viral particles replicating in host cells (Williams, 1998). Mechanisms of transmission for IIVs in YML include cannibalism, vertical transmission, or any cuticle-wounding activity (including other endoparasitoid, or nematode, entry; Williams, 2008). Data from other larval insects suggest ingestion is far less likely to cause disease than cuticular breach (Carter, 1974). Adults can also develop patent infections, showing characteristic bluish iridescence (Black et al. 1981; Kelly et al. 1979; Williams, 1998), but it is not known whether the virus can be contracted in the adult stage or if adult disease is due to carry-over from the larval stage.

All IIVs produce similar symptoms in larvae and patent infections are nearly always lethal. Upon infection, larvae become sluggish and develop iridescent coloration due to widespread viral replication in the epidermis and fat body (Williams, 1998, 2008). Larvae can live for several weeks after developing iridescence. Sublethal infections that do not result in widespread iridescence are thought to be common and likely affect growth and reproduction (reviewed in Ince et al. 2018). Asymptomatic or mildly symptomatic viral infections may go largely undetected in farmed insects until stressors instigate more widespread viral transmission or higher in-host replication, leading to epidemics (Fuxa et al. 1999; Weissman et al. 2012). While mortality from viruses is a clear measure of negative welfare resulting from disease, there is little known about the effect of mild infections on quality of life. Negative sublethal welfare impacts may be expected to result, minimally, from bodily damage due to viral replication or aforementioned behavioral restrictions (e.g., sluggishness, paralysis).

Two species of *Iridovirus* are well-known: IIV29 (Kelly et al. 1979) and IIV6 (Gençer et al. 2020). Only IIV29 has been detected circulating in natural populations, and transmissibility of IIV6 is quite low, indicating IIV6 may be present at very low levels (Bertola and Mutinelli, 2021; Gençer et al. 2020; Williams et al. 2005). IIV29 primarily causes pupal mortality, usually within a few days of puparium formation. A single pupa may host approximately 1.5 x 10^{10} IIV particles (Williams, 1998). Injected IIV6 particles generally cause mortality in the larval stage, with infected larvae becoming paralyzed three days after infection and dying prior to pupation (Gençer et al. 2020). However, IIV6 showed rather low infectivity in laboratory assays. Combined with the information that IIV6 has not been isolated from natural or farmed individuals, this suggests IIV6 is not currently a significant threat to farmed YM welfare.

Unfortunately, sampling for viral loads and prevalence of pathology in natural or farmed YMs is limited; therefore, the abundance and diversity of viruses, and pathogenicity of viral infections, remain largely unknown.

Bacteria

Insects form pathogenic (Bulla et al., 1975), commensalistic (Dillon and Dillon, 2003), and symbiotic (Gupta and Nair, 2020; Voirol et al. 2018) relationships with bacteria. Farmed YML have high bacterial loads by food hygiene standards (10^7 - 10^8 cfu/g; FASFC, 2014; Stoops et al. 2016; Vandeweyer et al. 2017), but the health and welfare consequences of such high loads are not known. The majority of insect pathogenic bacteria affect the larval stage, entering orally and crossing the intestinal barrier into the larval body, resulting in discoloration due to melanization (Bulla et al., 1975; Eilenberg et al. 2015; Nielsen-Leroux et al. 2012). However, these bacteria can also enter opportunistically through wounds in the cuticle (Bulla et al., 1975). Many pathogenic bacteria can persist outside of their host for at least a week (Dupriez et al. 2022; Eilenberg et al. 2015), allowing infections to periodically flare up when insects are stressed or facing multiple immune challenges.

YML are susceptible to several genera of pathogenic bacteria that can pose a threat to their welfare (or the welfare of the animals that eat them). *B. thuringiensis* is an entomopathogen and biocontrol agent that produces Cry3 toxins, which are lethal to YML (see section 'Cry Toxins'). The opportunistically pathogenic bacteria *Serratia marcescens* showed low virulence in YML but infections through the oral route still produced a small, significant increase in larval mortality over uninfected controls. *Serratia* persists in both feces and feed for over a week (Dupriez et al. 2022). The pathogenic bacteria *Pseudomonas aeruginosa* has also been detected in YM from commercial rearing facilities (Osimani et al. 2018). This species has been shown to increase cannibalism in larvae of the giant mealworm (*Zophobas morio;* Marciel-Vergara et al. 2018), suggesting it could increase the spread of other pathogens.

YML also shows susceptibility to known vertebrate pathogenic bacteria such as *Aeromonas hydrophilla* (Eilenberg et al. 2015) and *Salmonella* spp. (Jensen et al. 2020) in laboratory assays, but these have not been detected in farmed larvae to date (e.g., Garofalo et al. 2017; NVWA 2014; Vanderweyer et al. 2017). Krinsky (2019) notes that both larvae and adults inhabiting chicken farms are susceptible to *Salmonella* infection. Artificial contamination of feed with *Salmonella* spp., especially at high loads or for prolonged periods, can result in transfer to YML. To date, there are no reports of pathogenic effects that would be a welfare concern (Jensen et al. 2020; Wynants et al. 2019).

Gut microbiomes vary widely across insect taxa (Colman et al. 2012) and there is increasing evidence that the microbiome plays key roles in health, growth, and reproduction in farmed insects (Bruno et al. 2019; Jordan and Tomberlin, 2021). The majority of insect gut bacteria that are not cleared by immune system responses are considered commensalistic (no benefit /detriment to the host). However, such classifications are often revised to mutualisms when a benefit is discovered, suggesting a lack of research in this area limits informed classification of these relationships (and our interpretation of their welfare impacts; Lee et al. 2017).

The YML gut microbiota community shifts with changes in food source. Individuals reared in soil show greater microbiota biodiversity than controls reared in bran (Cambon et al., 2018; plastic also shifts gut microbiota, Lou et al. 2021). *Pediococcus pentosaceus* (Lactobacillales: Lactobacillaceae), related to a genus of lactic acid bacteria often associated with beneficial roles in vertebrate intestinal health (Dempsey and Corr 2022), along with three other species used as probiotics in vertebrates, *Bacillus subtilis*, *B. toyonensis*, and *Enterococcus faecalis*, significantly increased larval weight gain when provided as food additives (Lecocq et al. 2021; Rizou et al. 2022). The addition of these putative probiotic species to food also reduced the presence of bacteria in the order Enterobacteriales, which contains a number of known bacterial pathogens. *P. pentosaceus* isolates inhibited the growth of pathogenic bacteria (Lecocq et al. 2021), and improved larval growth and survival of YML infected with the pathogenic fungus *Metarhizium brunneum* (Dahal et al. 2022), suggesting this species might have antibiotic or antifungal properties.

Supplementing YML diets with probiotics may, through direct competition or by increasing nutrient availability, mitigate or prevent detrimental effects of pathogens, including any pain/discomfort and/or death associated with infections. If YML in mass-reared facilities are generally provided food without bacterial supplementation, even seemingly healthy individuals may be in substandard health. This suggests there is room to improve larval welfare, survival, weight gain, and health through probiotic supplementation. YML are coprophagous, and transmission of beneficial gut microbes through the consumption of conspecific feces may also benefit welfare (Weaver and MacFarlane, 1990).

Finally, although poorly studied, antibiotic treatment provided in feed may alter YM gut microbiota through reductions in bacterial load and/or diversity. Sterilizing gut microbiota caused reductions in body mass and survival, and early pupation for YML fed on certain substrates (Genta et al. 2006). Antibiotic use should thus be carefully considered as a treatment strategy for infection; further research would be required to assess the benefits and costs of antibiotic use for both economic performance and welfare in industry conditions.

Protists

Pathogenic protists are a common, though generally sublethal, problem for farmed YML (Eilenberg et al. 2015) and are a good target for regular monitoring efforts. In the middle of the 20th century large, commercial YM rearing facilities in Germany reported outbreaks of a pathogen that was likely protozoan. The pathogen existed as a moderate, non-fatal infection in 30–60 % of the population until more fatal disease outbreaks occurred. At times, outbreaks would be limited to very small areas of the rearing facility, suggesting that very localized environmental conditions or possibly cannibalism contributed to spread and increased parasitic load (Huger, 1967). A more contemporary survey of pet food stores and small-scale rearing facilities in central Europe reported *Cryptosporidium* from 12 % of facilities and *Gregarina spp.* from 33 % of facilities (Apicomplexa: Sporozoa; Gałęcki and Soko´ł, 2019).

These two protists are invertebrate-specific parasites that reproduce in the gastrointestinal tract of YML, often reaching very high densities and filling the host gut (Harry, 1967; Valigurová, 2007). Gregarines show a high prevalence in natural populations (Koura and Kamel, 1993) with species appearing to be somewhat life-stage specific (Clopton et al. 1993). Despite their prevalence, they do not generally cause disease in natural populations (Koura and Kamel, 1993) and may even be beneficial to some insects (Arcila and Meunier, 2020).

Experimentally-induced infections in YM adults and larvae are associated with reduced longevity (Rodriguez et al. 2007), though they are generally not considered highly lethal; it has been suggested they may also slow the host insect's movements and increase their susceptibility to other diseases (but data are needed to confirm these claims; Eilenberg et al. 2015). YML chronically infected with gregarines at low densities do not display the explicit negative effects often associated with welfare concerns, and which may manifest at higher densities (reduced health, reproduction, or longevity, or documented changes in behavior). However, even low densities may cause nutrient stress, which can induce negative effects on weight, development, and welfare via malnutrition (Harry, 1967).

Protists spread through cannibalism and via spores excreted into feed; proper nutritional provisioning and monitoring of YML density to prevent cannibalism, along with food sterilization, may help prevent outbreaks. Incubation of YML at 37.5 °C for six days rids them of *Gregarina*; however, this practice results in larval lethargy and approximately 10 % mortality, and therefore would not generally be advised from a welfare perspective. Heating bran feed prior to larval introduction significantly reduces *Gregarina* spore viability and subsequent infections of YML (MacDougall, 1942), offering a less invasive means of reducing outbreaks.

Amoebozoa and Coccidia infections appear to be rare, and are covered in Slowik et al. 2023.

Fungi

Most entomopathogenic fungi are necrotrophic parasites, killing their hosts in order to complete their lifecycle. YML are particularly susceptible to entomopathogenic fungi that occur naturally in soil (Eski and Murat, 2022; Keller and Zimmerman, 1989) and are even used as "bait insects" to collect *Beauveria spp*. and *Metarhizium spp*. from soil samples (e.g., Sharma et al. 2018; Vänninen, 1996); these genera are also both found colonizing stored grains (Slowik et al. 2023). These taxa breach the host cuticle using enzymes that weaken cuticular integrity and mechanical pressure to penetrate into the hemolymph (Aw and Hue 2017; Bidochka and Kharchatourians, 1994; Donatti et al. 2008). While both taxa are widespread in natural populations of YM (Praprotnik et al. 2021), only *B. bassiana* has been reported from commercial operations, where it increases mortality (Eilenberg et al. 2015).

In lab-based experiments on *Beauveria spp*. and *Metarhizium spp,* larval mortality rates range from less than 20 % to 100 %, depending on the fungal species and strain (Eski and Murat, 2022; Lestari and Rao 2016; Praprotnik et al. 2021; Shakeri and Foster, 2007). Adult mortality from *B. bassiana* is less severe, ranging from approximately 12 % to 75 % (Eski and Murat 2022). Fungal infections also appear to have non-lethal negative effects on reproduction. For example, while females do not discriminate between infected and uninfected males, healthy females that mated with infected males laid fewer eggs and/or fewer of their eggs hatched (Castillo-Peréz et al. 2022). Chronic/latent infections in production environments may thus depress colony-level reproductive output as well as harm the animals' welfare.

In farmed contexts, yeast and mold appear to be fairly common. Additionally, the human pathogenic yeast species *Candida albicans* and *C. neoformanshave* been found to cause mortality in *T. molitor* when directly injected into the haemocoel of the larvae (de Souza et al. 2015). For example, YML reared commercially for pet or human consumption show fungal loads of 10^4 –10^7 cfu/g (Stoops et al. 2016; Vandeweyer et al. 2017). In contrast, spices and dried nuts, which have some of the highest fungal loads in human foods, fall in a range from 10^1–10^4 cfu/g (Feroz et al. 2016; Berthold-Pluta et al. 2021). The relative magnitude of fungal loads are usually assessed from a perspective of human health and therefore welfare-related concerns for insects are not well characterized.

YML are exposed to mycotoxins from many fungi that grow in their plant-based diets (see section 'Mycotoxins'). The presence of fungi co-correlates with the presence of some species of commensal mites (it is unknown if the fungi drive the increase in mite prevalence, or if the same abiotic variables drive both fungi and mites to flourish concomitantly; Howard, 1955). Given their prevalence, fungi would be good candidates for regular monitoring programs in larger rearing facilities.

Arthropod parasites and parasitoids

The YM is host to a number of arthropod parasites, including nematodes, tapeworms, and ectopic arthropods. All may negatively affect YM welfare. Vertebrate intestinal nematodes have been reported from YM breeding facilities but instances are rare (Gałęcki and Soko´ł, 2019) and could possibly represent contamination from animals reared nearby for the pet trade rather than sustained infections. Insect-specific nematodes are often necrotrophic parasites, completing their life cycle within the dead host; therefore, infections generally result in high mortality rates. Much of our knowledge on nematodes' effects derives from research on two genera used for pest control, *Steinernema* and *Heterorhabditis* (Shapiro-Ilan et al. 2014). These genera harbor bacterial symbionts, *Xenorhabdus* in *Steinernema* and *Photorhabdus* in *Heterorhabditis* (Ciche, 2007; Ferreira and Malan, 2014), that they release into the insect hemolymph. The bacteria consume host resources as they develop and produce toxins that result in poisoning and death within 2–3 days (Cranshaw and Zimmerman, 2013). The nematodes then complete their life cycle within the body cavity of the dead host. Ultimately, the body wall of the host ruptures and emerging nematodes move short distances in the substrate to locate new hosts.

These nematodes are easily spread among species due to their broad host range and are expected to be of concern in any high-density and/or multi-species rearing facilities. *Steinernema* and *Heterorhabditis* enter host insects through the mouth, anus, or spiracles; *Heterorhabditis* can also enter through wounds in the cuticle (Ciche, 2007; Cranshaw and Zimmerman, 2013; Shapiro-Ilan et al. 2014). Infected YML experience significantly lower mortality when reared at 12 °C than 24 °C (however, this temperature is not practical for producers to employ long term given its effects on development, see section 'Temperature'; Susurluk, 2006). Increasing dietary lipid content from 5 % to 20 % results in a significantly higher mortality (Shapiro-Ilan et al. 2008).

T. molitor serve as an intermediate host of the rat tapeworm *Hymenolepeis diminuta*, consuming eggs that then hatch in the midgut and burrow into the haemocoel, forming cysticercoids. Exposure to eggs in food nearly always results in infection (Makki et al. 2011), making this a highly transmissible parasite. Still, since the YM serves as an intermediate host, infection rates appear to be quite low in the absence of infected rats (Makki et al. 2017). Infection by *H. diminuta* significantly reduced YM female fecundity (Hurd and Arme, 1986), but simultaneously increased longevity (Hurd et al. 2001). Importantly, overcrowding seems to enhance the negative effects on fecundity by reducing the number of eggs laid (Hurd and Arme, 1986). When infected, individuals showed lethargy, sensory issues (Hurd and Fogo, 1991), impaired defensive responses (Blankespoor et al. 1997) and slower learning in a T-maze (after two weeks; Sheiman et al. 2006), symptoms likely indicative of stress and reduced quality of life.

Importantly, because of the extension of lifespan, the negative physiological and behavioral effects of *H. diminuata* likely represent a prolonged state of stress for the YM. Although prevalence of *H. diminuata* in farmed YML is likely to be low, it presents several potentially welfare-relevant metrics of reduced quality of life that could be used to better understand the welfare implications of other interspecific interactions.

The YM is host to a number of ectoparasites composed largely of acariform mites (Class Arachnida, Superorder Acariformes). In a study of rearing facilities in central Europe, greater than 46 % of samples had Acariadae present (Gałęcki and Soko´ł, 2019). There is little information on the health or welfare effects of ectopic parasites. Commensal mites may also be present, feeding on the cereals inhabited by the YML (particularly when damp; see report of *Acarus siro* in Buxton, 1930); they can become detrimental to YMs when present in large numbers, generating competition for resources. In addition, when YML are injured (e.g., due to cannibalism), these mites migrate to and completely cover the open wound (Howard, 1955). The effect of this interaction on YML welfare is unknown.

YM pupae are also parasitized by a number of wasps including *Scleroderma guani*, *Chouioia cunea*, *Palmistichus elaeisis*. The ectoparasite *S. guani* first stings the pupa, injecting venom to immobilize it and suppress host immune responses (Wu et al. 2020a; Zhu et al. 2014), before laying its egg on the cuticle. Pupae stay alive for an average of 12.9 days following the injection of venom, during which time the wasp larvae hatches and begins to consume the living pupa (He et al. 2006).

Applications for producers: Disease

It will be important to consider effects on reproduction, behavior, and time to death when managing YM microbial interactions from a welfare perspective. As an overarching goal, the prevention of disease is desirable from both welfare and production standpoints. Therefore, many management practices that improve YM welfare will likely also bolster production.

Insect diseases are often instigated and exacerbated by poor hygiene and stress, with crowded conditions and nutritional deficits acting as underlying drivers (Eilenberg et al. 2015). Ensuring minimal opportunities for cuticular wounding and cannibalism may be especially important to preventing disease outbreaks. Further, low-density housing may actually bolster immune responses through low-level exposure. YML reared in small groups (15/400 cm^3 of food substrate; Table 3) exhibited a higher melanization response and experienced lower mortality upon exposure to the parasitoid fungus, *Metarhizium robertsii* than those reared individually (1/130 cm^3 of food substrate; Barnes and Siva-Jothy, 2000). Abiotic conditions can also be managed to reduce negative interspecific interactions. Fungal pathogens seem particularly responsive to humidity and maintaining YM colonies at lower humidity can deter fungal infections (Barnes and Siva Jothy, 2000; Eilenberg et al. 2018; see section 'Hydration').

While YML will eat discarded food scraps, farmed insects fed kitchen discards and locally collected feed sources showed higher infection rates of protozoan fungal pathogens than those fed only fresh products or specialized feed (Galecki and Sokok, 2019). This suggests that integrating a waste-as-feed model in rearing YM will likely exacerbate disease outbreaks. Pre-treatment of waste prior to feeding, e.g., via heating, could help kill fungi or protists that would harm YM welfare.

Additionally, there is a close relationship between nutritional status and immune system responses. Adult males with *ad libitum* access to food show greater PO activity and are more attractive to females (Rantala et al. 2003), whereas even short-term nutrient deprivation in adults can cause down-regulation of the immune system (Siva-Jothy and Thompson, 2002). Immune challenge also results in downregulation of metabolism and nutrient-storage genes (Johnson et al. 2014). Adequate nutrient provisioning should be provided alongside any procedural induction of the YML immune system to bolster resistance to pathogen outbreaks (see review of molecular mechanisms of immune response in Petronio et al. 2022).

In addition to taking preventative measures, producers may also monitor the "immune status" of their colonies by assaying blood-cell counts (haemocyte number), immune system responses such as antibacterial or phenoloxidase (PO) activity in the hemolymph (Laughton and Siva Jothy, 2011), or even using cuticular color as a coarse measure of pathogen resistance (Armitage and Siva Jothy, 2005; and other detection methods are described in Slowik et al. 2023). Rather than detecting a specific pathogen, this kind of monitoring would provide producers an index of the immune status of their colonies (Armitage and Siva Jothy, 2005). Low-level infections may go undetected for long periods of time until conditions such as crowding, high humidity, or weakened immune function facilitate outbreaks. It is thus especially important to monitor for disease-causing agents regularly, even when symptomatic individuals are not present.

Finally, producers may take steps to bolster the immune status of their YM colonies through immune priming. While bacterial or fungal overgrowth are problematic, a sterile environment should not be the aim as the YM immune system is bolstered by low-level exposure to immune challenges. Producers can facilitate this through logistic choices such as low stocking densities or through intentional immune priming via mild exposure to pathogens, toxins, etc. For example, preexposure of YML to bacterial toxins appears to induce a long-term antimicrobial response that enhances survival (Moret and Siva Jothy, 2003). While immune priming requires exposing YM to mild stress, the short term welfare challenge may be outweighed by the long term welfare benefits of improved immune function. Immune priming can also be transgenerational such that exposure in the adult stage induces greater immune response in offspring (reviewed in Kojour et al. 2022). This may be beneficial from a welfare perspective, as exposing a smaller number of breeding adults to a mild stressor produces a large number of offspring who have not experienced that stressor but are nevertheless better protected against disease.

Supplementation with probiotics may also have benefits to YM welfare. While more research is needed on the probiotic capacity of specific bacteria for YM, supplementation with known vertebrate probiotics enhanced weight gain and even reduced some pathogenic bacteria (Lecocq et al. 2021; Rizou et al. 2022). Anti-parasitic tannins derived from plants have been shown to reduce tapeworm infections in YM (Dhakal et al. 2015) and could, in combination with close monitoring of colony health, be utilized on an as-needed basis. More details on these and other farmed insect disease prevention measures, especially in relation to production facility infrastructure and hygiene practices, can be found in Eilenberg et al. (2015, 2018), Joosten et al. (2020), Slowik et al. (2023), and International Platform of Insects as Food and Feed (2022).

Genetic modification, selective breeding, and inbreeding depression

Genetic modifications are being explored for farmed YM, including the use of CRISPR-Cas9 (Chu, 2019) and RNA interference (RNAi; Oppert et al. 2019), which may involve rendering specific (presumably undesirable) genes non-functional or introducing novel (presumably desirable) genes from hetero- or conspecifics (reviewed in Errikson and Picard, 2021). These manipulations were made possible in part by the publication of the YM genome

(Eriksson et al. 2020). The most likely genetic modifications explored for YM are those that would increase economic productivity, for example by: increasing growth rates or feed utilization, speeding up reproduction or development, increasing survival or fecundity, or generating better disease resistance. Undesirable characteristics, such as traits that increase allergenicity (Ribiero et al. 2021) may also be targeted for removal. Genetic modification could provide another avenue for extending developmental period to increase larval weights (e.g., as in black soldier fly larvae; Zhan et al. 2019), instead of the current practice of incorporating juvenile hormone in the diet (see section 'Hormonal Additives').

As an example of the complex effects that may result due to genetic modification, consider the recent use of RNAi to knockdown YM *vermilion,* producing white eyes in *T. molitor*. *Vermilion*-knockdown in YMs affected the expression of 109 other genes, including a reduction of Aspartate 1-decarboxylase (ADC). Decreased ADC expression in flour beetles resulted in behavioral defects, including a reduction in movement speed and distance and an increased amount of time spent immobile (Perkin et al. 2017). *Vermilion* mutant fruit flies had increased longevity compared to wild-type (Oxenkrug, 2010) but saw age-dependent memory failure (Savvateeva et al. 1999). Genetic modifications may thus have complex, unintended physiological or behavioral consequences that manifest as animals age (Dennis, 2002). Accordingly, any genetic modifications should be studied for their progressive impact on welfare across the lifespan, prior to large-scale adoption by the industry. Importantly, YM *vermilion* knockdown required individual injection of dsRNA, and resulted in only 48 % survival of treated-embryos (compared to 59 % in mock-treated controls) and 70 % efficacy at the larval stage, so it is unlikely to be viable for the industry at scale (Oppert et al. 2019).

Selective breeding (i.e., artificial selection) to enhance production is perhaps of more immediate interest to the industry (Tomberlin et al. 2022). Selective breeding has historical precedent and can be done particularly efficiently in r-strategist species (life history characterized by high fecundity, small body size, short generation time, etc.) like the YM (Eriksson and Picard, 2021). Strains within rearing facilities are already differentiating phenotypically (presumably, due to both natural and artificial selection, as well as neutral evolutionary processes), with documented variation in final larval body mass, development time, survival, and feed utilization (Adamaki-Sotiraki et al. 2022; Rumbos et al. 2021). Alongside further selective breeding to increase the prevalence or magnitude of economically valuable phenotypes, this variability may allow for hybridization between strains to enhance productivity (Eriksson and Picard, 2021).

Two artificial selection experiments (7–8 years long) selected for larger late-stage YM larval body mass, reduced development times, greater conversion efficiency of ingested food, and higher egg production (LeClercq, 1963; Morales-Ramos et al. 2019). However, the aforementioned benefits were counteracted by reduced survival in the selected strain, resulting in similar biomass production overall. Larval survival was 10.5 % in the selected strain and 24.2 % in the ancestral strain by 60-69 days. Approximately 9 % of the artificially selected larvae died *after* six weeks of development (Morales-Ramos et al. 2019), suggesting the negative physiological states that are eventually lethal in this strain may be experienced for a prolonged duration prior to mortality. It is thus possible that trait tradeoffs (e.g., between larval survival and mass) may provide no benefit to economic productivity while harming welfare.

However, there is also an opportunity for selective breeding to enhance farmed YM welfare. For example, selection for 'black' lines of YM adults correlated with improved disease resistance (Armitage and Siva-Jothy, 2005; Armitage et al. 2003). Selective breeding fixed the 'black' cuticular color in YML over three generations with only a slight reduction in late-stage larval mass (Song et al. 2022; and see Huang et al. 2011). Selective breeding could thus improve insects' resistance to immune challenges faced in high-density farmed conditions, with concomitant benefits for welfare. Nevertheless, selective breeding has been responsible for many welfare problems in vertebrate livestock production (Paxton et al. 2013). Researchers and producers should take care to evaluate the welfare impacts of any selective breeding initiatives in their facilities.

Finally, inbreeding depression may occur during artificial selection processes (e.g., when effective population sizes of breeding adults are likely to be smaller), or accidentally whenever continuously breeding closed populations of captive insects (Eriksson and Picard, 2021; Gilchrist et al. 2012). Inbreeding can result in reduced growth, morphological defects, decreased longevity, and reduced stress or disease resistance (reviewed in Jensen et al. 2017), which may result in colony collapse and should thus be avoided for welfare and economic reasons. Inbreeding for even a single generation in YM reduced adult mass and pre-adult survival, increased developmental time, and reduced resistance to *B. bassiana* fungi (Rantala et al. 2011). Male pheromonal attractiveness is also affected negatively by inbreeding which may affect colony level reproductive output (Pölkki et al. 2012). Reintroducing genetic diversity to reduce the negative welfare consequences of inbreeding may occur through strain hybridization or crossing with wild populations (Jensen et al. 2017).

Morphological defects

Morphological defects in the adult life stage result from inappropriate pupation. The prevalence of morphological defects in YM populations is not well reported; one lab study reports 4 % of adults are defectively formed (Errico et al. 2021). Deformation in insects may result from disease (e.g., deformed wing virus in honey bees), genetics, or abiotic factors. For example, storage of pupae at 4 °C resulted in 100 % deformity of the wing or elytron of eclosed adults (Patterson and Duman, 2001). High larval rearing densities also led to the retention of pupal characteristics into adulthood, compared to lower densities; this may be the result of consuming compounds with juvenile hormone activity found in the frass of conspecifics (Weaver and McFarlane, 1990). The welfare effects of deformation are not well understood; some defects may restrict natural behaviors like mating or burying, or cause/contribute to mortality.

Cannibalism

YML may cannibalize other larvae or pupae, or even dying/injured adults when reared together. Pupae may be particularly vulnerable due to their relative immobility. When physical defense mechanisms against conspecific cannibalism were removed in YM pupae, the majority were cannibalized within six hours and nearly all were killed within 2–3 days (Ichikawa and Kurauchi, 2009). In fact, cannibalism in this group can be such a significant risk to pupal survival that it may have contributed to the evolution of wandering behaviors in the beetles' last larval stage (Tschinkel, 1981). The presence of larval conspecifics can thus inhibit pupation in Tenebrionid beetles (Connat et al. 1991; Tschinkel and Willson, 1971). Separating larvae and pupae (Morales-Ramos et al. 2012), as well as 'young' and 'old' larvae (Zega Enterprises, 2020), is important to protect the vulnerable groups. However, age-matched larvae may also cannibalize one another (Weaver and McFarlane, 1990).

The driving factor(s) of cannibalism in YML are not established. Providing a water source has been suggested to help prevent cannibalism (Hardouin and Mahoux, 2003).

Nutritional inadequacies (e.g., when fed styrofoam or plastics, see Yang et al. 2021b, and video by sigma1920HD, 2022, also Mlček et al. 2021) may also contribute to cannibalism. Starved larvae had a cannibalism rate of 11 % - 37.2 %, while larvae fed only plastics had a cannibalism rate of 5.3 % - 23.0 %, compared to 1.7 -14.9 % on a bran diet (Yang et al. 2021a,b).

High larval rearing density may increase cannibalism irrespective of abundant nutrition, especially at later larval stages (Weaver and McFarlane, 1990; but see Zaelor and Kitthawee, 2018 where no effect was found). As density increased from 2 to 20 individuals/455 ml rearing jar, survival to pupation declined from 94 % to 47.5 % (and to adulthood, 94 % to 32.4 %). Cannibalism was only observed at the higher densities, and may occur when larvae are molting too close to conspecifics in crowded conditions (Weaver and McFarlane, 1990). In other cannibalistic insect larvae reared in artificial environments, regular crowding and malnutrition led to the evolution of increased cannibalistic behavior across generations (Vijendravarma et al. 2013). Maintaining high-quality larval nutrition and low rearing densities could assist the industry in avoiding accidental selection for increasingly cannibalistic, and thus economically unviable, strains. Differences in rates of cannibalistic behavior among studies may already indicate such strain-level variation exists.

Additionally, greater substrate depth could help separate larval instars by development/size, as the earlier instars have been reported to be more positively geotactic (Cloudsley-Thompson, 1953). If behavioral responses to gravity result in reduced physical proximity of bigger and smaller larvae when given more depth, this could also reduce larval cannibalism. Unreported variation in substrate depths could also account for some of the differences in cannibalism rates among studies.

Notably, cannibalism may not result in the immediate death of the cannibalized individual; instead, larvae or pupae may simply be injured but remain alive for hours (or days; Ichikawa and Kurauchi, 2009) prior to succumbing to hemolymph loss or putative septicemia (Weaver and McFarlane, 1990). Non-lethal wounds caused by cannibalism breach the cuticle, with holes that may be several mm in diameter (Weaver and McFarlane, 1990), and thus may also increase instances of disease (see section ‘Physical Health and Interspecific Interactions’). Cannibalism may thus result in prolonged periods of injury and/or disease before death in YM and should be considered a significant welfare concern.

Adults may also cannibalize eggs, particularly as rearing density increases (Morales-Ramos et al. 2012). Producers should manage adult densities to avoid this phenomenon, as it reduces colony level productivity (Deruytter et al. 2019; see section ‘Rearing Density - Adults’). Available data suggest adult cannibalism of other adults is not as common. However, adults of other Tenebrionidae are likely to cannibalize newly eclosed individuals, especially when conditions are dry (Tschinkel and Willson, 1971). Mounting an immune response to cuticular wounding was found to be energetically costly in adult YM beetles, suggesting injuries from cannibalism may negatively affect reproductive output (Ardia et al. 2012).

Behavior

Notably, behavioral restriction and expression have already been covered throughout many of the previous sections (e.g., preference of high- vs. low-humidity microclimates when YM larvae or adults are/are not provided with moisture sources; see section ‘Hydration’). Below, we briefly cover a few behaviors not covered explicitly prior to this section.

Adult mating and oviposition needs

Relatively little is known about the factors that promote natural mating and oviposition behaviors of YM adults. A feeding and oviposition substrate is required for survival and oviposition (adults failed to lay in empty tubes or on muslin). The minimum temperature for oviposition was found to be 14 °C (Dick, 1937). As already reviewed elsewhere in this paper, the abiotic variables that ensure high adult survival in the provided substrate should be met to protect both welfare and reproductive output.

Females are naturally polyandrous (as well as engaging in repeated matings with individual males). Continuous mating opportunities with multiple males should be provided, which will also materially and/or genetically benefit colony-level reproduction (Drnevich et al. 2001, Font and Desfilis, 2003; Worden and Parker, 2001). Old feeding substrates may not be ideal for supporting female oviposition; in studies of other Tenebrionidae (*Tribolium castaneum*) oviposition rates decline where feces and, potentially, defensive secretion accumulate while nutrients deplete (Romero, 2007, Romero et al. 2010).

Restricting pupation

Rearing at high densities (see section ‘Rearing Density - Larvae’) or with hormonal additives (see section ‘Hormonal Additives’) reduces YM pupation rates. Pupation is a natural part of the YM lifecycle; rearing at such high densities that pupation is repressed may present a behavioral restriction. This is especially the case when YML have already entered the ‘wandering’ phase of development.

Handling-associated stress

Farmed YM may be handled during the rearing process to:

1. Maintain the colony (e.g., remove dead individuals or change food/water sources)
2. Check health and development
3. Process and slaughter
4. Transport

Some handling is necessary to maintain high welfare conditions (e.g., changing substrates to provide quality nutrition or checking for signs of disease). Stress should be minimized by reducing the frequency of handling and avoiding stress-inducing practices whenever possible. Increased handling was hypothesized to reduce growth and survival in Deruytter et al. (2019). However, the removal of larval frass (as part of their experimental design) cannot be ruled out as the causative agent, as YML are coprophagous and may get essential gut microbes from the consumption of conspecific frass, particularly as young larvae.

Natural behaviors may serve as a guide in determining the handling practices that may stress YM. For example, adult and larval YM are photophobic (see section ‘Light’). Therefore, it may be especially important to minimize exposure to stressful light conditions during handling, for example by using red lights.

Additionally, predators are linked to stress hormone release in many insects (Cinel et al. 2020), resulting in a suite of evolved anti-predator behavioral responses, such as tonic immobility (Humphreys and Ruxton, 2018). Handling that is superficially similar to predator threats, such as being physically handled or the feeling of vibrations in the substrate, and may cause stress.

Indeed, experimenters physically 'jarring' the experimental arena to cause vibrations resulted in tonic immobility in adult YM beetles (Krams et al. 2013a). The presence of a nocturnal predator (whose activity caused vibrations in the substrate) increased hiding behaviors and tonic immobility in adult YM (Krams et al. 2013b). YM adults with increased individual resting metabolic rates had reduced tonic immobility latency and durations (Krams et al. 2013a, 2013b), and differences in anti-predator responses were highly repeatable for individuals (Krams et al. 2014). This may reflect consistent individual differences in internal motivational or affective states. Vibrations are linked to stress hormone levels in adult insects of other taxa (Adamo and Baker, 2011). YM adults may acclimate to handling with more frequent handling events, as expressed by a reduced duration of tonic immobility (Howard, 1955). In any case, frequency and duration of tonic immobility could be assessed as potential indicators of welfare status in farmed YM adults during handling.

Increased stress in response to handling has not been studied in YML, though 'violent locomotion' is reported in response to rough handling (see discussion in Cloudsley-Thompson, 1953). However, given that anti-predator adaptations are common in insect larvae as well as adults (e.g., Ramirez et al. 2010; Sih, 1986), further research may find a useful behavioral indicator of welfare during handling by looking at YML anti-predator adaptations.

Pre-slaughter handling, fasting, and washing

Pre-slaughter handling may prove to be a particularly stressful experience for YML, as vibrating sieves may be used to separate YM from the feed (see section 'Processing for Slaughter & Transport'). It's unclear if the vibrations produced by machinery are experienced similarly to the vibrations produced by predators that can cause stress in insects. Still, vibrations and noise from nearby machinery were posited as a potential reason for slow larval growth in Li et al. (2015).

Processing of larvae prior to slaughter may involve washing and fasting for ~48 hours, in theory to remove microbial contaminants (van Huis, 2021). However, Wynants et al. (2017) demonstrated that contamination levels in YML did not change as a result of either practice (similar results were obtained for black soldier fly larvae, Larouche et al., 2019). Given avoidance of high-moisture environments, being washed or dipped in water may cause stress. Fasting may negatively affect welfare through hunger/starvation or injury induced by cannibalism (see section 'Cannibalism'). Given the short duration of fasting, and high desiccation tolerance of YML, it is unclear if fasting would induce hydration-related welfare challenges.

In addition, YML may also experience stress due to being out of their feeding substrate for a prolonged period of time during the pre-slaughter handling and fasting stages, restricting their natural burrowing and photophobic behaviors. Behavioral observations of late-instar YML extracted from their feeding substrate show that they engage in a rapid searching behavior along the edge of the arena and, if provided with substrate, will immediately bury themselves again even if the substrate provided is not especially nutritive (Barrett, pers. comm). Given that wandering insect larvae are at high risk of predation in the wild (Aluja et al. 2005), the tendency for YML to quickly re-bury themselves may be to avoid predation. Continuing to wander for a prolonged period in search of a substrate represents an energetic cost to the animal and may indicate that the denial of burying opportunities and/or lack of nutrition during fasting is stressful for YML.

Given that contamination levels do not appear to change as a result of washing or fasting, it may be possible to largely discontinue the use of these practices, improving production efficiency while simultaneously improving YML welfare. Of course, prior to discontinuing these practices, further tests with different rearing substrates, microbial loads, and environmental pollutants would be necessary to confirm the effect of removing washing and fasting steps on product safety and quality.

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