Effect of temperature and photoperiod on development, survival and growth rate of mealworms, *Tenebrio molitor*

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Ich erkläre zusätzlich unbefangen den Versuch und das Verfassen dieser Masterarbeit, unabhängig genannter Unternehmen, durchgeführt zu haben.

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Abstract

A growing world population and their growing demand for meat will have negative climatic and environmental impacts. Insects are a possible substitute and part of a sustainable human diet due to their valuable nutrients and relatively low environmental production impact. One of the species which are already produced for human consumption is the mealworm, i.e. larvae of *Tenebrio molitor*. Knowledge of temperature and photoperiod on mealworm development is scarce. Therefore, the effect of three temperature (20 °C, 25 °C and 30 °C) and three photoperiod regimes (LD 16:8, SD 8:16 and constant darkness) were tested on survival, developmental time and growth rate, to detect the most efficient rearing conditions. There is a significant effect of temperature on survival rate, developmental time and growth rate, and a significant effect of photoperiod on developmental time and growth rate of mealworms. At 30 °C and constant darkness there is the highest survival and highest growth rate, and the shortest developmental time. Concluding, temperature and photoperiod are major factors in rearing *T. molitor* and optimising these factors is important for an efficient production.

Keywords: entomophagy, mealworm, *Tenebrio molitor*, temperature, photoperiod, farming, rearing

Zusammenfassung

Eine wachsende Weltbevölkerung und deren steigende Nachfrage nach Fleisch wird negative Auswirkungen auf das Klima und die Umwelt haben. Durch ihre wertvollen Nährstoffe und ihre relativ geringen Umweltauswirkungen bei der Produktion sind Insekten ein möglicher Ersatz und Bestandteil einer nachhaltigen menschlichen Ernährung. Eine der Arten, die schon jetzt für den menschlichen Verzehr produziert wird, ist der Mehlwurm, die Larve von *Tenebrio molitor*. Über den Einfluss von Temperatur und Photoperiode auf die Entwicklung des Mehlwurms ist wenig bekannt. Deshalb wurde der Einfluss von drei Temperaturstufen (20 °C, 25 °C und 30 °C) und drei Photoperioden (Langtag 16:8, Kurztag 8: 16 und durchgehende Dunkelheit) auf das Überleben, die Entwicklungszeit und die Wachstumsrate der Mehlwürmer getestet, um die effizientesten Zuchtbedingungen festzustellen. Die Temperatur hat einen signifikanten Einfluss auf die Überlebensrate, Entwicklungsdauer und Wachstumsrate, und die Photoperiode hat einen signifikanten Einfluss auf die Entwicklungsdauer und Wachstumsrate. Bei 30 °C und durchgehender Dunkelheit besteht die größte Überlebens- und Wachstumsrate und die kürzeste Entwicklungsdauer. Folglich sind Temperatur und Photoperiode wesentliche *T. molitor*-Zuchtfaktoren und für eine effiziente Produktion ist es wichtig diese Faktoren zu optimieren.

Stichwörter: Entomophagie, Mehlwurm, *Tenebrio molitor*, Temperatur, Photoperiode, Landwirtschaft, Zucht

1. Introduction

As the world population and the demand for conventional meat is expected to increase (Godfray et al. 2010, Alexandratos and Bruinsma 2012), negative climatic and environmental consequences are expected to follow (Foley et al. 2011, Tilman and Clark 2014). The current meat production is responsible for 14-51 % of anthropogenic greenhouse gases (McMichael et al. 2007, Oonincx et al. 2010), 70% of agricultural land use (Steinfeld et al. 2006), 29% of the agricultural water footprint (Mekonnen and Hoekstra 2010) and biodiversity loss (Crenna et al. 2019). A sustainable diet can mitigate negative effects on climate and environment, for example by incorporating insects (Oonincx and de Boer 2012, van Huis et al. 2013, Joensuu and Silvenius 2017, van Huis and Oonincx 2017). There are more than 2,000 known insect species (over 30% Coleoptera) suited for human consumption, i.e. (anthropo-) entomophagy, with a high nutritional value (Finke 2002, Rumpold and Schlüter 2013, Jongema 2017, Raheem et al. 2019). Most likely insects were part of early humans diets (Ramos-Elorduy 2009, van Itterbeeck and van Huis 2012) and worldwide more than two billion people eat insects regularly, mostly by harvesting wild insects (DeFoliart 1999, van Itterbeeck and van Huis 2012, Jongema 2017, Feng et al. 2018). In Europe there are few insect species which are consumed by humans (Jongema 2017), for example *Tenebrio molitor* (Coleoptera: Tenebrionidae) (Makkar et al. 2014).

Insect farming for human consumption only began recently (van Huis et al. 2013) and is a growing economic sector and field of research (Müller et al. 2016, Madau et al. 2020, van Huis 2020). But this sector also needs to overcome some obstacles. One being consumer acceptance (Mancini et al. 2019b), as there is a disgust factor which hinders Europeans' willingness to adopt insects into their diet (Lammers et al. 2019), with different strategies to overcome this issue. For example, by education, incorporating insects into products and gastronomic interest (Deroy et al. 2015, Collins et al. 2019). Others are ethical considerations (Gjerris et al. 2016), food safety aspects and legislation issues for insects as food and feed (BMGF 2017, Raheem et al. 2019, Cappelli et al. 2020). Since 2018, EU regulation 2015/2283 regulates edible insects and products derived from insects which need safety assessments before market approval (Raheem et al. 2019).

Tenebrio molitor is already widely used for food and feed production (Makkar et al. 2014, Morales-Ramos et al. 2019), studied as model organism (Adamski et al. 2019) and has many rearing advantages (Hein 1924). There are publications and patents describing a commercial

mealworm production (Ghaly and Alkoaik 2009, Dossey et al. 2016, Kröncke et al. 2020, van Huis 2020). In Austria there are already insect farms, producing e.g. mealworms (larvae of *T. molitor*), and derived products for human consumption and feed. WURMFARM, for example, is a commercial mealworm production and research partner for this thesis, located in Carinthia and LIVIN FARMS is a commercial mealworm production based in Vienna and Hong Kong. ZIRP is an insect retailer based in Vienna.

1.1. Why to eat mealworms?

There are two main reasons why humans might eat insects. First, their nutritive value and second, their resources-efficient production. Depending on species, developmental stage, sex, feed composition, farming technology, conditions and processing there are varying nutrient compositions of insects (Rumpold and Schlüter 2013, Siemianowska et al. 2013, Simon et al. 2013, Oonincx et al. 2015, van Broekhoven et al. 2015, Adamkova et al. 2016, Nowak et al. 2016, Payne et al. 2016, Adamkova et al. 2017). Table 1 shows mealworm nutrients depending on feed, rearing conditions and calculation method (Nowak et al. 2016, Jonas-Levi and Martinez 2017). Mealworms contain high amounts of protein with all the essential amino acids to meet human dietary requirements (Ghaly and Alkoaik 2009, Bednarova et al. 2013, Yi et al. 2013, Zielinska et al. 2015, Adamkova et al. 2016, Azagoh et al. 2016, Nowak et al. 2016, Zhao et al. 2016, Poelaert et al. 2018). They have a high energy content, up to 552 kcal per 100 g dry weight (Raheem et al. 2019), resulting from a high fat content (Cito et al. 2017) (Table 1) which consist of favorable fatty acids, e.g. mono- and polyunsaturated acids, like oleic and linoleic acid, essential components of human nutrition (Siemianowska et al. 2013, van Broekhoven et al. 2015, Zielinska et al. 2015, Adamkova et al. 2016, Zhao et al. 2016). They also contain vitamins, e.g. B-12 (Finke 2002, Rumpold and Schlüter 2013, Baek et al. 2019, Mlcek et al. 2019), high amounts of valuable minerals, e.g. Zn, Fe, Cu, Mg and Mn (Finke 2002, Rumpold and Schlüter 2013, Siemianowska et al. 2013, Zielinska et al. 2015, Mwangi et al. 2018) and chitin (Marono et al. 2015). Mealworms contain several micro- and macronutrients which can have health benefits, as lowering cardiovascular disease risk (Dreassi et al. 2017, Mlcek et al. 2019), prevention of oxidative stress-related diseases (Siemianowska et al. 2013, Tang et al. 2018, Baek et al. 2019, Mancini et al. 2019, Son et al. 2020) and anti-inflammation activity (Son et al. 2020). Edible insects have a good digestibility (Ramos-Elorduy 1997, Manditsera et

al. 2019) and are a promising ingredient and supplement in human diets (Rumpold and Schlüter 2013, Cito et al. 2017, Poelaert et al. 2018, Baek et al. 2019, Mlcek et al. 2019).

Table 1 Nutritional composition of fresh weight and dry matter mealworms (larvae of *T. molitor*), described in weight percentages (%), divided in moisture, carbohydrates CH, protein, fat, fiber and ash. Depending on rearing conditions, feed (incl. water source) and calculation method.
Finke 2002, Ghaly and Alkoaik 2009, Li et al. 2013, Rumpold and Schlüter 2013, Siemianowska et al. 2013, Oonincx et al. 2015, van Broekhoven et al. 2015, Zielinska et al. 2015, Adamkova et al. 2016, Zhao et al. 2016, Bjorge et al. 2018, Poelaert et al. 2018, Mancini et al. 2019, Melis et al. 2019, Zhang et al. 2019, Liu et al. 2020, Rumbos et al. 2020.

Basis	Moisture (%)	CH (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)
Fresh weight	56.27-69.8	2.73-12.54	10.15-27.6	6.93-21.93	5.7	0.9-1.55
Dry matter		2.2-37.5	28.41-76.2	6.44-50	1.97-20.22	2.36-8.14

However, mealworms can cause allergic reactions via ingestion, inhalation or skin contact (Garino et al. 2019). Cross-reactivity can occur if people are allergic to dust mites and crustacea (Verhoeckx et al. 2014, van Broekhoven et al. 2016, Barre et al. 2019). This requires appropriate worker safety conditions (Cappelli et al. 2020) and labelling of mealworm products (Barre et al. 2019).

There are additional risks, especially high microbial loads (Vandeweyer et al. 2017, Garofalo et al. 2019, Cappelli et al. 2020), which make compliance with food safety regulations (e.g. cleaning the production facility and application of Hazard Analysis and Critical Control points (HACCP) mandatory, and processing and frequent testing of mealworms advisable (Eilenberg et al. 2015, Eilenberg et al. 2018, Raheem et al. 2019, Wynants et al. 2019). To reduce microbial loads there are different production, cooking (e.g. boiling, blanching and fermentation), extractions (e.g. fractionation and drying) and storage methods (e.g. freezing) which affect extraction and solubility (Zhao et al. 2016, Yi et al. 2017, Janssen et al. 2019), moisture content (Azzollini et al. 2016), nutrients, their functionality (Lenaerts et al. 2018, Poelaert et al. 2018, Purschke et al. 2018, Tang et al. 2018, Baek et al. 2019), digestibility and bioaccessibility (Megido et al. 2018, Manditsera et al. 2019) and microbial loads and shelf life (Klunder et al. 2012, Rumpold et al. 2014, Borremans et al. 2018, Megido et al. 2018, De Smet et al. 2019, Mancini et al. 2019, Murefu et al. 2019, Cappelli et al. 2020).

There are many products which can be derived from mealworms, for example oil (Zhao et al. 2016, Son et al. 2020), paste/gel (Yi et al. 2013, De Smet et al. 2019) and powder (Azzollini et al. 2016, Kröncke et al. 2020, Son et al. 2020). *Tenebrio molitor* frass has versatile applications, e.g. as biofertilizer (Poveda et al. 2019, Yang et al. 2019b, Houben et al. 2020).

Mealworms are also used as feed for pets (e.g. mammals, birds, reptiles, amphibians and spiders) (Cotton 1927, Finke 2002, Feng et al. 2018, Mariod 2020), fish (Henry et al. 2015, Azagoh et al. 2016), poultry (Bovera et al. 2016, Selaledi et al. 2019) and pigs (Jin et al. 2016).

Concerning the production of insects, they have several advantages compared to other livestock. Insects, being poikilothermic, have a high feed conversion rate which enables an efficient transformation of a relative low amount of feed into edible biomass (van Broekhoven et al. 2015, van Huis and Oonincx 2017, Bjorge et al. 2018). Mealworms can be reared on regional food by-products and food waste which are not in competition to human food. This enables a transformation of waste into nutritious food or feed, socioeconomic benefits, self-subsistence in low income regions and lowers the cost of farming mealworms (Xu et al. 2013, Oonincx et al. 2015, van Broekhoven et al. 2015, Alves et al. 2016, Mancini et al. 2019, Yang et al. 2019, Zhang et al. 2019). They can even be farmed in closed artificial agricultural ecosystems in extreme environments, which are not suited for traditional agriculture, and in space to provide astronauts with food (Li et al. 2013, Li et al. 2016).

An additional advantage of a mealworm production, compared to conventional meat production, is the relatively low environmental impact (Oonincx and de Boer 2012, Miglietta et al. 2015, Joensuu and Silvenius 2017). A life cycle analysis found that one kg of edible mealworm protein has a global warming potential of 14 kg of CO_2-equivalent and uses 18 m^2 of land (Oonincx and de Boer 2012), compared to 45-643 kg of CO_2-equivalent and 37-2100 m^2 for one kg of edible beef protein (Nijdam et al. 2012, Flachowsky et al. 2017). Twenty-three liters of water are required to produce one g of mealworm protein (Miglietta et al. 2015) but 112 liters for one g of beef protein (Mekonnen and Hoekstra 2012). The required farming energy is similar (de Vries and de Boer 2010, Oonincx and de Boer 2012). Other conventional farming animals require less resources, compared to beef, but there are also additional emissions and pollutions, e.g. acidification (NH_3 emissions) (de Vries and de Boer 2010).

Figure 1.1 WURMFARM production plastic trays with different *T. molitor* developmental stages with rearing substrate in different trays (Koitz and Schaden s.a.).

Substituting conventional animal products with insects results in a resource saving potential, less land use, mitigation of greenhouse gases and reduced pressure on biodiversity (McMichael et al. 2007, Mekonnen and Hoekstra 2010, Oonincx et al. 2010, Oonincx and de Boer 2012, Machovina et al. 2015, Abbasi et al. 2016, Govorushko 2019). Considering that the mealworm production and therefore its productivity and upscaling is still in an early stage, this potential can even be greater (Oonincx and DeBoer 2012) (Figure 1.1). To farm efficiently, it is important to know the most important farming conditions and their effect on *T. molitor* life cycle and physiology.

1.2. Biology of *T. molitor*

Tenebrio molitor is a holometabolous, synanthropic, omnivore pest species feeding on stored products. This species has a cosmopolitan distribution, except for the "oriental region" (i.e. India, Indonesia and Philippines), and lives mainly in temperate regions, e.g. in granaries, under rocks, logs or in animal burrows (Hill 2003, Robinson 2005, Löbl and Smetana 2008, Gullan and Cranston 2010).

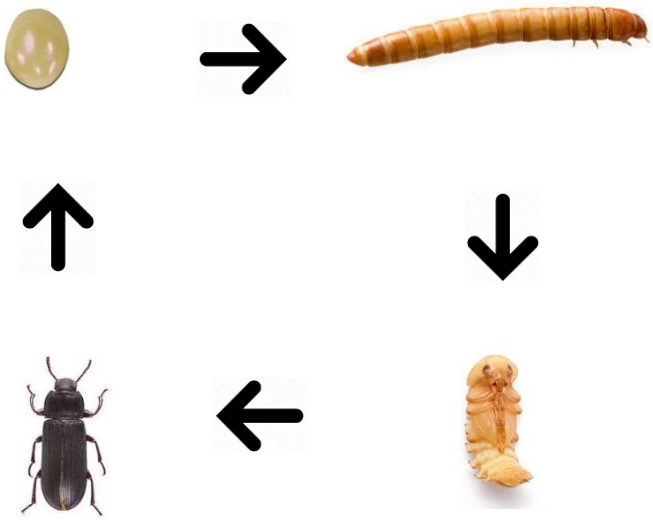

Figure 1.2 Life cycle of *Tenebrio molitor*. Egg (upper left), larvae also called mealworm (upper right), pupae (bottom right) and adult beetle (bottom left). Depiction is not proportionate.

Eggs (Figure 1.2) are ovoid, white, translucent, soft (Balfour and Carmichael 1928, Punzo and Mutchmor 1980) and adhered to small particles because they are covered with a sticky secretion (Robinson 2005). Eggs do not survive at temperatures below 12.5 °C and above 35 °C (Kim et al. 2015) and at a relative humidity of 12% or lower (Punzo and Mutchmor 1980). Larvae hatch after 5-9 days at 25-35 °C and 50-70% relative humidity (Ludwig and Fiore 1960, Huang et al. 2011, Kim et al. 2015) and after about 40 days at 15 °C (Ludwig and Fiore 1960, Kim et al. 2015).

Freshly hatched and molted larvae are whitish and soft. After hardening they become yellow-brown (Figure 1.2) or even blackish (Balfour and Carmichael 1928, Hill 2003, Huang et al. 2011). They are cylindrical, slender, waxy, well sclerotized (Balfour and Carmichael 1928,

Robinson 2005), up to 30 mm in length (Punzo and Mutchmor 1980, Hill 2003, Kröncke et al. 2020) and weigh 80-200 mg before pupation (Connat et al. 1991, Ghaly and Alkoaik 2009, Mariod 2020). Their developmental time is 76-629 days (Cotton 1927, Morales-Ramos et al. 2010). Larvae have twenty-three sensilla on maxillary palps and thirteen sensilla on labial palps used for olfactory, gustatory functions and chemical communication, with no differences among instars, suggesting a uniform feeding habit and lifestyle (Ruschioni et al. 2019). Mealworms have a great developmental plasticity concerning instar number and instar length, which are influenced by intrinsic factors, like juvenile hormone and ecdysteroids and extrinsic environmental factors (Cotton and St. George 1929, Connat et al. 1991, Morales-Ramos et al. 2010, Morales-Ramos et al. 2015). Generally, instar number increases with adverse conditions (Esperk et al. 2007 cited in Morales-Ramos et al. 2010). They molt between 9-23 times (Cotton 1927, Ludwig 1956), with 14-17 molts being average (Park et al. 2014, Morales-Ramos et al. 2015). The first instar, with a developmental time of 3-4 days, and the last instar before pupation are of similar length within the same diet (Morales-Ramos et al. 2010, Park et al. 2014). Instar 1-4 are a fixed part of the mealworm life cycle (Morales-Ramos et al. 2010). Stadium length is positively correlated with total number of instars starting from instar 5. There is a low stadium length variability in instars 5-9 and stadium length increases continuously between instar 10 and the last instar, which means that most variability and instar insertion seems to happen late in larval development (Morales-Ramos et al. 2010, Morales-Ramos et al. 2015). Body length increases with each instar (Park et al. 2014). Head capsule width and mandible size are variable within instar and together with larval weight are overlapping between instars, which limits the application of Dyar's Law to predict instars (Morales-Ramos et al. 2015).

Murray (1968) observed two growth phases of mealworms characterized by molts. Larvae increase in weight linearly until a few days before molting (phase 1). There is only little weight increase or even weight loss before molting because of frass passage, and in newly molted larvae because of an adjustment period and evaporative water loss until the cuticle is hardened (phase 2) (Murray 1968). At the end of larval development they can even decrease in weight (Urs and Hopkins 1973, Ghaly and Alkoaik 2009, Morales-Ramos and Rojas 2015) which is in contrast to numerous other insects, which feed the most before pupation (Connat et al. 1991).

Mealworms are positively thigmotactic and tend to aggregate in large cultures, moist spots and warm places, negatively phototactic, negatively geotactic and most active during the night (Cotton and St. George 1929, Cloudsley-Thompson 1953, Punzo 1975, Weaver and McFarlane

1989). Large larvae tend to live near the surface and sometimes come to the surface at night. Young larvae live in deeper layers (Cloudsley-Thompson 1953).

Larval survival and developmental time (and therefore growth rate as calculated in this thesis) are influenced by temperature (Ludwig 1956, Punzo and Mutchmor 1980, Koo et al. 2013, Bjorge et al. 2018), photoperiod (Tyshchenko and Ba 1986 cited in Ribeiro et al. 2018, Kim et al. 2015), water availability (Murray 1968, Urs and Hopkins 1973, Punzo and Mutchmor 1980, Oonincx et al. 2015, Rumbos et al. 2020), parental age (Ludwig 1956, Ludwig and Fiore 1960, Ludwig and Fiore 1961), instar number (Morales-Ramos et al. 2010), genetics (Urs and Hopkins 1973), larval color (Huang et al. 2011), population density (Connat et al. 1991, Weaver and McFarlane 1990, Zaelor and Kitthawee 2018), oxygen concentration (Loudon 1988, Greenberg and Ar 1996) and feed (Morales-Ramos et al. 2010, Oonincx et al. 2015, van Broekhoven et al. 2015, Kim et al. 2016, Kim et al. 2017, Zhang et al. 2019, Liu et al. 2020, Rumbos et al. 2020). These are important farming conditions and are described in more detail in the following chapters.

Tenebrio molitor pupae are white and turn yellowish in a later phase (Figure 1.2). The pupal stage lasts for 6 or 48 days at 30 °C and 15 °C, respectively (Ludwig and Fiore 1960, Robinson 2005) and is shorter if larvae are reared with a water source (e.g. in feed or high relative humidity) (Urs and Hopkins 1973). The pupal stage is the most resistant to temperature, they can survive at 4 °C for 15 days, and to relative humidity extremes, 12% and 98% (Bowler 1967, Punzo and Mutchmor 1980, Sönmez and Koc 2019). They are immobile with an abdominal rotation as defense against cannibalism (Ichikawa and Kurauchi 2009).

Adults hatch whitish-brown and the cuticle turns to dark-brown or black (Hill 2003, Robinson 2005) (Figure 1.2). They have short and thick antennae (Hill 2003) and their thorax is punctured, their forewings are longitudinally straited, but they usually do not fly (Robinson 2005). Their body is dorsoventrally flattened to be able to dig into the substrate, to avoid high temperatures (Punzo 1975). Adults live between 60 and 125 days (Ludwig and Fiore 1960, Hill 2003) depending on environmental conditions and sex (Urs and Hopkins 1973, Punzo 1975, Rho and Lee 2016).

Adults are attracted by pheromones from the other sex (Bryning et al. 2005) and begin to mate 2-7 days after eclosion (Gerber 1975, Spencer and Spencer 2006). Males can assess female reproductive status via chemical cues and transfer a spermatophore to females (Drenvich et al. 2000, Carazo et al. 2004). Females are continuously receptive, prefer healthy males (e.g. not

infected with the tapeworm *Hymenolepis diminuta*) and mate multiple times with several males (polyandry) to produce more offspring (Worden et al. 2000, Drenvich et al. 2001, Worden and Parker 2005). Females start to lay eggs 4-17 days after mating (Gerber 1975, Mariod 2020). They can lay up to 40 eggs per day (Robinson 2005) or up to 500 in their lifetime (Hill 2003, Mariod 2020). They oviposit beneath the surface layer, prefer moist habitats and dig deeper at high adult densities and when eggs or larvae are present (Cloudsley-Thompson 1953, Punzo and Mutchmor 1980, Gerber and Sabourin 1984). Oviposition, fecundity and progeny number are also influenced by adult population density (Morales-Ramos et al. 2012, Berggreen et al. 2018, Deruytter et al. 2019), adult age (Morales-Ramos et al. 2012), environmental conditions (Punzo and Mutchmor 1980) and feed (Rho and Lee 2016, Rumbos et al. 2020). *Tenebrio molitor* beetles are nocturnal and come to the surface at night (Balfour and Carmichael 1928, Cloudsley-Thompson 1953).

1.2.1. Influence of temperature on development

Insects are poikilothermic and therefore their metabolism depends on ambient temperature. Van't Hoff's equation states that the speed of enzymatic reactions increases with increasing temperature (Mortimer and Müller 2007). Temperature therefore influences survival (Punzo and Mutchmor 1980), developmental time (Ludwig 1956, Koo et al. 2013, Kim et al. 2015), growth rate (Bjorge et al. 2018), nutrient composition (Adamkova et al. 2017, Bjorge et al. 2018), reproductive physiology and behavioral characteristics of mealworms (Punzo 1975). Thus, among the most important factors to control for an efficient commercial production, developmental time, costs and product quality (Bjorge et al. 2018). The upper lethal temperature of *T. molitor* larvae is 42-44 °C (Mellanby 1954 cited in Bowler 1967, Altman and Dittmer 1973, Allen et al. 2012). The chill-coma temperature, the low temperature threshold below a response to stimuli fails to occur, is below 7-12 °C, depending on exposure time and cold or warm adaptation (Mutchmor and Richards 1961). Larvae can survive at 4 °C for several weeks but lose mass and are freeze-intolerant (Cotton and St. George 1929, Graham et al. 2000). In cold climates, mealworms overwinter in a quiescent state (Cotton and St. George 1929, Qin and Walker 2006). The temperature optimum of *T. molitor* is between 22 and 28 °C (Mellanby 1932 and Howard 1955 cited in Punzo and Mutchmor 1980, Spencer and Spencer 2006). To avoid adverse temperatures, mealworms express behavioral thermoregulation, e.g. digging into substrate, orientation towards or away from a heat source and seeking favorable microhabitats,

and physiological thermoregulation, e.g. waxy layers, evaporation control and utilization of metabolic heat (Punzo 1975, Gullan and Cranston 2010). The hemolymph of mealworms contains an antifreeze protein which produces a thermal hysteresis, a temperature difference between the freezing and melting points, which depresses lower lethal temperatures (Patterson and Duman 1978, Qin and Walker 2006).

Ectotherm species respond to rapid temperature changes by increasing or decreasing their metabolism and rely on behavioral adjustments to avoid e.g. high temperatures (Punzo 1975, Allen et al. 2012). At these temperatures animals are incapable of coordinated movement which is also influenced by ambient oxygen concentrations (Stevens et al. 2010, Allen et al. 2012). Critical thermal minimum stays at about 4 °C, when exposed to 15 °C prior and increases to about 6 °C, when exposed to 35 °C for at least one day. Critical thermal maximum stays at about 43.5 °C, when exposed to 15 °C prior and increases from 44 °C to about 45 °C after a two-day exposure to 35 °C (Allen et al. 2012).

Punzo and Mutchmor (1980) tested *T. molitor* egg, larva, pupa and adult survival at different temperatures and humidities. They state that 25 °C represents an optimal temperature for a high survival rate and a non-stressful condition for larvae, as there occurred no deaths under moist conditions (52% and 75% relative humidity) and very little mortality under relative humidity extremes (12% and 98%). Temperatures of 10 °C and 35 °C constitute stress conditions as the survival rate decreases especially under dry or very moist conditions (Punzo and Mutchmor 1980). The older the larvae, the higher its survival time at critical temperatures (Punzo 1975). Temperatures above 37 °C inhibit larval growth (Punzo and Mutchmor 1980, Bjorge et al. 2018). It is important to consider factor interactions between temperature and humidity as the temperature has a more severe limiting effect at extreme relative humidity conditions and vice versa (Punzo and Mutchmor 1980). Koo et al. (2013) and Kim et al. (2015) found significantly different mealworm developmental times at different temperatures with the fastest development of about 111 days at 30 °C and about 127 days at 27,5 °C respectively. Ludwig (1956) found shorter developmental times at 25 °C with approximately 150 days, compared to 30 °C with 160-213 days, however, detailed statistical analyses were lacking.

At 31 °C Bjorge et al. (2018) recorded the highest mealworm wet mass growth per day and the highest metabolic rate, but the highest energy conversion efficiency occurred at 23.3 °C. Water, protein and lipid contents also depend on rearing temperature with the highest lipid and the

lowest protein content at 31 °C, compared to other temperature regimes (Adamkova et al. 2017, Bjorge et al. 2018).

Mealworm farming at high densities needs to consider a possible increase of temperature by metabolic heat production, as mealworms tend to aggregate (Cloudsley-Thompson 1953, Weaver and McFarlane 1989) which can have adverse effects on development, survival and growth rates. Depending on the farmer's objective (short developmental time, high feed conversion rate or specific nutrient composition) there are different temperature recommendations which are between 23 °C and 31 °C (Koo et al. 2013, Kim et al 2015, Adamkova et al. 2017, Bjorge et al. 2018).

1.2.2. Influence of photoperiod on development

Insects use photoperiod to measure and respond to day/night length for diapause induction and termination, for phenological, developmental and behavioral adjustments (Saunders 2012). After photoreception and measurement of day/night length, inductive photoperiods are accumulated which leads to a release or retention of neurohormones regulating a diapausing or non-diapausing development (Saunders 2014).

Tenebrio molitor does not enter diapause, but quiescence to overcome unfavorable environmental conditions (Chippendale 1984 cited in Qin and Walker 2006). *Tenebrio molitor* (adults and larvae) is negative phototactic and inhabit dark environments, e.g. burrow in granaries/substrate, influenced by the reaction to light and gravity, and adults or old larvae come to the surface at night, depending on light intensity (Cloudsley-Thompson 1953, Yinon 1970). Under constant photoperiodic conditions the diurnal periodism of movement and rest can disappear and populations can become arrhythmic (Cloudsley-Thompson 1953). Their 24-hour rhythm correlates with light and darkness and is independent of temperature (Cloudsley-Thompson 1953), but the interaction of photoperiod and temperature is important considering the development of insects (Lopatina et al. 2011, Saunders 2014). Photoperiod can modify the thermal reaction norm of insect development, resulting in a change of thermal developmental threshold and thermal requirements for development, depending on day length (Lopatina et al. 2011). Kutcherov et al. (2018) exemplifies this by demonstrating an accelerated larval development at long-day conditions, compared to short-day conditions, with a stronger developmental response at low temperatures. In the tested summer active species, *Scantius*

aegyptius (Hemiptera: Pyrrhocoridae), the accelerated long-day development maybe allows a completion of an additional generation in favorable summer conditions and in the tested spring active species, *Timarcha tenebricosa* (Coleoptera: Chrysomelidae), the same photoperiodic response maybe ensures a completion of the larval stage, which is vulnerable to heat (Kutcherov et al. 2018). To what extend this applies to *T. molitor* stays a question for future research, especially for mealworm populations which live in constant environmental conditions (e.g. farms and granaries) and maybe already adapted their developmental response to a constant temperature and photoperiod/darkness. This makes the source of mealworm strains an important information for mealworm farmers, as this potential adaptation can lead to different photoperiod farming conditions for different mealworm strains for an efficient production.

Ribeiro et al. (2018) state that photoperiod influences mealworm development and growth but do not specify this effect (Tyshchenko and Ba 1986 cited in Ribeiro et al. 2018). Kim et al. (2015) found significant shorter mealworm developmental times and significant longer pupal periods under long-day conditions (14 L, 10 D). Photoperiod also influences eclosion rates with the lowest eclosion rate under 10 L, 14 D (Kim et al. 2015).

1.2.3. Influence of water on development

Tenebrio molitor, a xeric species, is resistant to desiccation and adapted to dry environments (Urs and Hopkins 1973). Nocturnal activity and the adaptation of trachea size are linked to water conservation and low levels of sodium in the hemolymph results in a reduced water loss via metabolic rate and respiration (Punzo 1975, Gullan and Cranston 2010).

Mealworms take up water via drinking, feed (also hygroscopic effect of carbohydrates and proteins) and metabolic processes. A net gain of water uptake from feed occurs at a relative humidity of 70% or above (Machin 1975). In the cryptonephric system, mealworms can absorb water from the atmosphere (Ramsay 1964, Dunbar and Winston 1975, Machin 1975, Machin 1976, Coutchie and Machin 1984, Hansen et al. 2006). They can lose water through excretion, respiratory exchange and cuticular water loss (evapotranspiration), depending on permeability and temperature of the cuticle (increased loss after molt until hardening of cuticle) and water vapor pressure gradient (Murray 1968, Punzo and Mutchmor 1980, Gullan and Cranston 2010).

Increased water uptake and/or availability increases survival (Punzo and Mutchmor 1980, Morales-Ramos et al. 2010, Oonincx et al. 2015, van Broekhoven et al. 2015, Rumbos et al. 2020) decreases developmental time (Urs and Hopkins 1973, Morales-Ramos et al. 2010, Oonincx et al. 2015, van Broekhoven et al. 2015) and increases weight and therefore growth rate (Fraenkel et al. 1950 cited in Machin 1975, Murray 1968, Urs and Hopkins 1973, Morales-Ramos et al. 2010, Oonincx et al. 2015, van Broekhoven et al. 2015, Liu et al. 2020).

A relative humidity of 52% or 75% has no detrimental effect on larval survival, even at extreme temperatures (10 °C or 35 °C). Dry or very moist conditions, with 12% and 98% relative humidity, result in a higher mortality at extreme temperatures (Punzo and Mutchmor 1980). But the interaction of temperature and humidity is important to consider as environmental factors are rarely independent from each other. Humidity exerts a more severe limiting effect at extreme temperature conditions and vice versa (Punzo and Mutchmor 1980). For example, at 35 °C and dry conditions increased evaporation leads to a lower survival of mealworms and at the same temperature regime but a relative humidity of 98% survival of old mealworms is reduced because of a limited cooling effect through limited evapotranspiration (Punzo and Mutchmor 1980). Mealworms can go in a dormant state to reduce water loss and therefore survive unfavorable conditions (Murray 1968). Fraenkel et al. (1950 cited in Machin 1975) showed that mealworm growth rates increased at relative humidities between 30% and 70%.

For mealworm farming, a relative humidity of 70% is recommended (Spencer and Spencer 2006). At this point there is a balance between water intake and loss (Machin 1975) and above 70% is an increased chance of mold occurrence. To increase farming efficiency, it is recommended to supply a water source, e.g. via cotton pads or fresh feed (e.g. salad, cabbage, carrots, apples) (Urs and Hopkins 1973, Morales-Ramos et al. 2010, Oonincx et al. 2015, van Broekhoven et al. 2015).

1.2.4. Influence of feed on development

Diet affects mealworm survival, developmental time, growth rate, feed conversion efficiency, number of instars, length of instars, nutrient composition (dry matter, carbohydrate, amino acids, fatty acids, ash content), microbial loads as well as pupal and adult traits (Morales-Ramos et al. 2010, Morales-Ramos et al. 2013, Oonincx et al. 2015, van Broekhoven et al. 2015, Kim et al. 2016, Li et al. 2016b, Dreassi et al. 2017, Kim et al. 2017, McConnell and Judge 2018,

Mancini et al. 2019, Melis et al. 2019, Mlcek et al. 2019, Stull et al. 2019, Zhang et al. 2019, Liu et al. 2020, Rumbos et al. 2020). Diets rich in protein (e.g. yeast from brewer's spent grains), depending on quantity and source, increase survival and feed conversion efficiency, decrease developmental time and alter carbohydrate, protein and fat composition of mealworms (Morales-Ramos et al. 2013, Oonincx et al. 2015, van Broekhoven et al. 2015, Kim et al. 2016, Dreassi et al. 2017, Kim et al. 2017, Mancini et al. 2019, Melis et al. 2019, Rumbos et al. 2020). Diets high in starch content are correlated with a high larval biomass (Rumbos et al. 2020) but the protein/starch ratio and their sources are important to consider for an optimal development (van Broekhoven et al. 2015). Through diet manipulation and supplementation mealworms nutritional compositions can be altered to desired nutrient compositions, e.g. fatty acid ratios (n3/n6 ratio) and specific health benefits (Klasing et al. 2000, Siemianowska et al. 2013, Zhao et al. 2016, Mlcek et al. 2019, Zhang et al. 2019, Son et al. 2020).

Depending on the feed and its source, cultivation method, processing and mealworm starvation before harvest (Wynants et al. 2017, Garofalo et al. 2019) there is a risk of parasitic transfer (Galecki and Sokol 2019), organic contaminants (e.g. pesticides) transfer (Houbraken et al. 2016, Poma et al. 2017, van Broekhoven et al. 2017, Niermans et al. 2019), bacteria, e.g. *Salmonella* sp. retention (Wynants et al. 2019) and bioaccumulation of heavy metals, e.g. Hg and As (van der Fels-Klerx et al. 2016, Poma et al. 2017, Truzzi et al. 2019).

1.2.5. Influence of larval density on development

The density of a farming population is an important factor to control for an efficient biomass output of mealworms and a high number of offspring for future generations (Morales-Ramos et al. 2012, Morales-Ramos and Rojas 2015, Berggreen et al. 2018, Zaelor and Kitthawee 2018, Deruytter et al. 2019). If different *T. molitor* life stages are not separated there is a high chance of cannibalism with increasing density (Weaver and McFarlane 1990, Ichikawa and Kurauchi 2009, Deruytter et al. 2019). Despite mealworms' tendency to aggregate, influenced by lactic acid, present in mealworm frass (Cloudsley-Thompson 1953, Weaver and McFarlane 1989), larval density and food conversion efficiency are negatively correlated. The higher the density, the smaller the food conversion efficiency and growth of mealworms (Weaver and McFarlane 1990, Morales-Ramos and Rojas 2015).

There is an inhibition of pupation at high densities (Tschinkel and Willson 1971) and for mass production an adult density of 8.4 adults per dm^2 is most efficient (Morales-Ramos et al. 2012).

1.2.6. Influence of other farming conditions on development

There are several other farming conditions which influence mealworm survival, development and growth. Ludwig and Fiore (1960, 1961) found at 20 °C and 25 °C larvae from one month old parents developed significantly faster, than larvae from freshly emerged adults. Hypoxia conditions increases larval mortality and developmental time (Loudon 1988, Greenberg and Ar 1996, Gullan and Cranston 2010).

In a closed farm, the main entry route of mealworm pathogens is human activity and feed (Eilenberg et al. 2018). Edible insects can be natural carriers of antibiotic resistant bacteria which can be dangerous to human health (Osimani et al. 2018). Viral diseases (Maciel-Vergara and Ros 2017) and fungal pathogens which infest feed can reduce survival and growth of *T. molitor* (Guo et al. 2014). Increased fitness and a reduced microbial load can be achieved by quantitative nutritious feed, supplementation of immune activity producing feed additives, probiotic bacteria in feed, antibiotics, direct immune priming, transgenerational immune priming, the selection of darker beetles and processing (Rantala et al. 2003, Morales-Ramos et al. 2013, Grau et al. 2017, Osimani et al. 2017, Vigneron et al. 2019). Trans-generational immune priming occurs in *T. molitor*, as an immune challenge in parents induces production of antimicrobial peptides in the hemolymph of their offspring (Moret 2006). Trans-generational or direct immune priming can therefore increase immunity in the farming population (Vigneron et al. 2019). Cuticular melanization, influenced by temperature, density and food, is closely linked to immune response (Krams et al. 2016, Vigneron et al. 2019). Darker beetles have a thicker and less porous exocuticle (Silva et al. 2016), increased phenoloxidase activity, enhanced hemocyte concentration (Armitage and Siva- Jothy 2005) and are more resistant to fungal diseases (Barnes and Siva-Jothy 2000). Temperature also plays a significant role in mealworm immune response, as immune response of lipopolysaccharide-challenged mealworms correlates positively with preferred body temperature (Catalan et al. 2012).

Morales-Ramos et al. (2019) selected artificially for large-size pupae for eight years and received a mealworm strain with increased size, growth rate and fecundity, but also a lower survival rate. Urs and Hopkins (1973) found significantly different developmental times

between different strains of mealworms, indicating a genetic influence. Huang et al. (2011) found different developmental times and feed conversion efficiencies in two colored varieties of mealworms. This leads to the assumption that artificial selection could result in a higher biomass productivity (Morales-Ramos et al. 2019).

Insect farming is considered as breeding of common livestock and therefore must follow rules of animal welfare (EC No. 1069/2009). Animal welfare implies a fulfilment of material and immaterial conditions which are preconditions for animal health and in accordance with its environment (Dolezal et al. 2004 cited in Adamkova et al. 2017). One method to comply with animal welfare is abidance of Webster's five freedoms of animal welfare (Webster 2016). De Goede et al. (2013) state that there is no consensus about insect pain perception. First steps to comply with pain minimization is, e.g. killing by freezing, which is the natural way many insects die in nature (Lenaerts et al. 2018) and results in no nutritional stress as an indicator of welfare (Adamkova et al. 2017).

2. Aims of this thesis

To increase farming efficiency, research for optimal farming conditions is essential. *Tenebrio molitor* is widely used for food and feed production (Makkar et al. 2014), but some optimum rearing conditions are still not researched in detail. The effect of temperature on important farming parameters is known (Ludwig 1956, Bowler 1967, Punzo and Mutchmor 1980, Koo et al. 2013, Kim et al. 2015, Adamkova et al. 2017, Bjorge et al. 2018), but the knowledge of how different photoperiod regimes affects important farming conditions is lacking. This thesis aims to shed light on the effect of temperature and photoperiod on mealworm development. Three different temperature (20 °C, 25 °C and 30 °C) and photoperiod (long day LD 16:8, short day SD 8:16 and constant darkness D 24:0) regimes were tested to confirm the effect of temperature and to clarify the effect of photoperiod on survival rate, developmental time and growth rate of mealworms, by answering the following research questions:

A) What is the optimum temperature for a high survival rate, low developmental time and high growth rate of mealworms?

B) What is the optimum photoperiod for a high survival rate, low developmental time and high growth rate of mealworms?

Additionally, the genetic structure of the *T. molitor* strain used in this thesis was determined, to help future research to compare the genetic variance and origin of their *T. molitor* strains.

3. Material and Methods

3.1. Stock culture

Tenebrio molitor used in this study originated from the WURMFARM, Bad Sankt Leonhard, Carinthia, Austria.

On 16 July 2018 freshly hatched adult beetles were transported from WURMFARM to IFFF-BOKU. The stock culture was maintained at 22 °C, natural light, a relative humidity of approximately 30%, in a plastic box with aeration slits and egg cartons for hiding purposes and filled with about 2 cm of feed. The nutritional content of this feed, which was also used throughout the study is listed in Table 3.1. Additional feed was added once feed got sparse and pieces of carrots were added to provide moisture about once per month throughout the stock culture maintenance.

Table 3.1 Nutritional content of feed used in this study – same as the one used by WURMFARM. Nutrients described in weight percentages (%), divided in carbohydrates CH, protein, fat and fiber. Vitamins: A, B1, B2, B3, B5, B6, B7, B9, B12, C, D, E, folate; minerals: Na, Mg, K, Ca, Mn, Fe, Cu, Zn, P, J, Se, S; choline; biotin; inositol are without indication of quantity.

	Weight (%)	**CH (%)**	**Protein (%)**	**Fat (%)**	**Fiber (%)**
Lucerne	8	0.9	0.7	0.1	2.1
Maize meal	12	8.3	2	0.5	0.3
Beer yeast	10	3.1	4.8	0.4	1
Wheat bran	70	12.1	11.2	3.3	31.6
Sum	**100**	**24.4**	**18.6**	**4.3**	**34.9**

3.2. Preparation of experiments

For subsequent experiments on *T. molitor* development Table 3.2 gives an overview of the chronological procedures of experiments. Between 17 and 19 July 2018 female and male *T. molitor* adults, randomly selected from the stock culture, were put in plastic boxes (9x6x5 cm)

with lids having aeration holes to initiate mating and oviposition. Beetles were sexed based on differences in the abdominal sternites (Bhattacharya et al. 1970). Due to uncertainties in this determination, most boxes were filled with mating beetle pairs randomly selected from the stock culture. Each box was filled with one cm of feed and fifteen boxes were put in each of the nine incubators (details on experimental setup see below) (Figure 3.2). Oviposition was finished about three weeks later and adults were removed. Five boxes were marked and microscoped daily to screen for hatched larvae. Larvae (4^{th} to 6^{th} instar) in these boxes were used to define the starting point of the experiment (Appendix 1). This also enabled an undisturbed development of hatched larvae in the other ten experimental boxes.

Table 3.2 Procedure of experiment in chronological order, arranged in timeframes and corresponding tasks.

Timeframe	Task
13.7. - 15.7.2018	*Tenebrio molitor* beetles of stock culture hatched
17.7. - 12.8.2018	Oviposition period of adults and hatching period of larvae
13.8. - 21.8.2018	Larvae development until instar 4-6
22.8.2018 - 7.6.2019	Data collection with weekly analysis of larvae

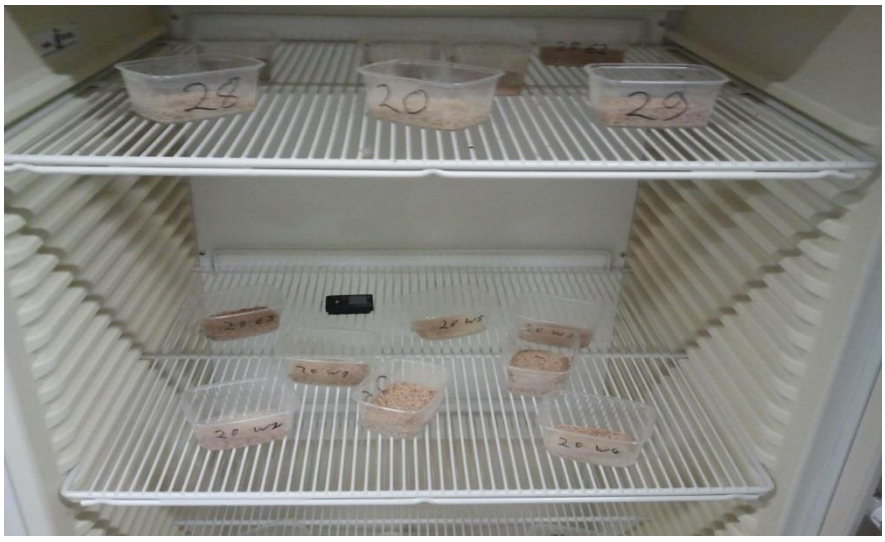

Figure 3.1 One of nine incubators used, with boxes containing larvae and feed: Boxes were placed randomly in incubators and a data logger (testo 174 T ®, black device in picture) was used to monitor temperature und relative humidity.

3.3. Experimental trials

To identify the effect of temperature and photoperiod on survival, development and growth of larvae of *T. molitor*, three temperature regimes, 20 °C, 25 °C and 30 °C and three different photoperiods, i.e. long day LD (16 light L, 8 dark D), short day SD (8 L, 16 D) and permanent darkness 24 D were selected. There were nine different experimental conditions. Per condition there were ten plastic boxes (n = 10, Table 4.1, 4.2, 4.3) and 20 larvae per plastic box.

Between 22 August and 15 September 2018 larvae were separated from their feed (Figure 3.3) and separated according to their instar determined by their head capsule width according to Morales-Ramos et al. (2015) (Figure 3.4). To measure their head capsule width, each larva was placed under a microscope with an ocular micrometer 10x21, calibrated with an object micrometer. To keep larvae inactive, they were placed on ice (Figure 3.4). Twenty 4^{th} to 6^{th} instar larvae with head capsules between 494.6 ± 34 and 658.5 ± 51.8 µm (Table 3.3) were randomly selected, their total weight was measured with a Mettler Toledo MT5 scale and put in boxes filled with one cm of fresh feed. Thus, density reached roughly 0,37 larvae per cm^3 or 2,7 cm^3 feed per larvae. 4^{th} to 6^{th} larval instar as starting point for assessment of developmental time and survival rate was chosen because at this point larvae were visible and distinguishable from feed which made separation from feed, handling and weighing easier. Dead larvae and pupae were removed from experimental boxes weekly, to record survival rate. Furthermore, weekly weighing was performed to calculate growth rate and measurement of head capsule width to monitor instar development. This process was repeated for each box once a week until pupation (Appendix 1 and 2). Feed was added twice on 28 October 2018 and on 04 May 2019. Data collection was finished on 07 June 2019 (Table 3.2).

The data loggers *testo 174 T*® were used to monitor temperature and relative humidity. The maximum temperature fluctuation was ± 2 °C (Appendix 3). On 01 October 2018 plastic boxes (9x6x5 cm) filled with water were placed in all 25 °C and 30 °C incubators to adjust the relative humidity to a similar level. On 20 November 2018 the same was done for all 20 °C incubators to increase relative humidity from 20-30% to about 50% with fluctuations of ± 5% (Appendix 3). At this point larvae in 20 °C incubators reached 9^{th} to 11^{th} instar and had similar average weights of 10-20 mg as larvae in 25 °C and 30 °C incubators on 1 October 2018.

Table 3.3 Head capsule width in µm ± SD of *T. molitor* larvae by instar, starting at the third instar (Morales-Ramos et al. 2015).

Instar	Head capsule width (µm)
3	439 ± 16.4
4	494.6 ± 34.5
5	575.7 ± 41.6
6	658.5 ± 51.8
7	758.2 ± 81.1
8	868.6 ± 96.6
9	1006.2 ± 120.8
10	1191.1 ± 155.3
11	1398.8 ± 194
12	1642.6 ± 236.2
13	1873 ± 256.5
14	2113.6 ± 258
15	2294.6 ± 257.2
16	2377.9 ± 213.3
17	2254.6 ± 149.8
18	2343.8

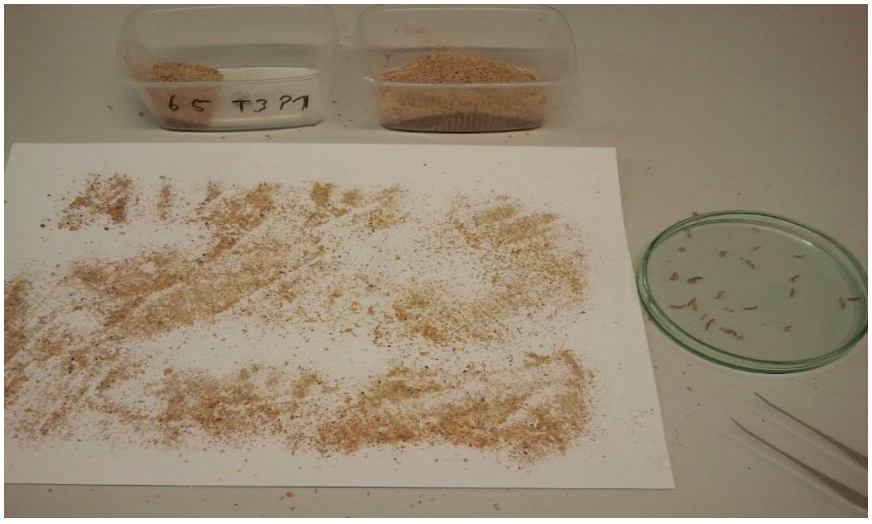

Figure 3.2 Separation of larvae from feed by emptying each box on a fresh sheet of paper. Larvae were put in Petri dishes with fine forceps.

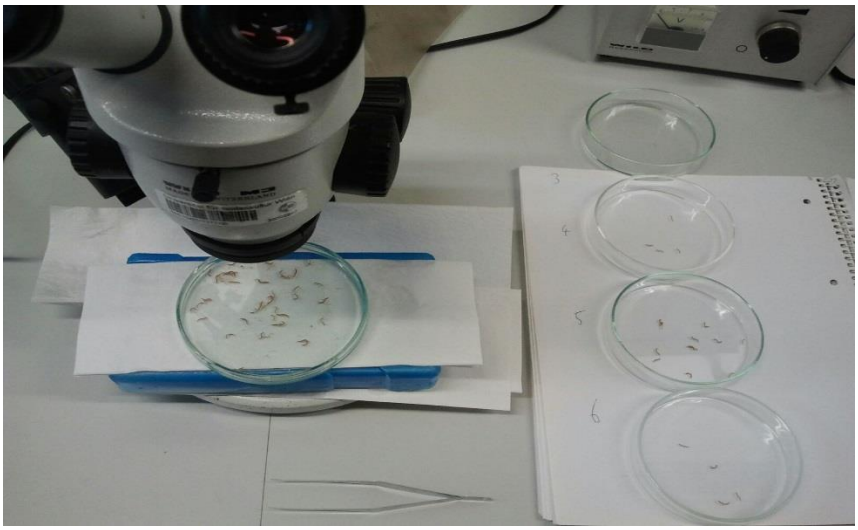

Figure 3.3 Measurement of head capsule width with WILD Heerbrugg microscope with an ocular micrometer 10x21, calibrated with an object micrometer. Larvae were separated on ice (blue) according to their instar (Table 3.3).

3.4. Data analysis

To test the effect of temperature and photoperiod on the development of mealworms, a univariate ANOVA was performed for survival rate, developmental time and growth rate. Tukey-test was used as a post-hoc-test to test for significant differences between factor levels. Additionally, a non-parametric Kruskal-Wallis-test, was performed in case of a deviation of ANOVA assumptions. Alpha was set at 0.05. All analyses were conducted with SPSS version 24.

Survival rate: Survival rate was recorded from 4^{th} to 6^{th} instar until pupation. Survival rate was calculated per box. Larvae which did not pupate during data collection or were lost during data collection were dismissed as outliers in the statistical analysis (Appendix 2). The number of all pupated larvae divided by the number of larvae at the beginning of the experiment minus outliers defined the survival rate per box (n).

$$n = \frac{\sum \text{pupae}}{20 - \text{outliers}} * 100 \text{ (in \%)} \quad (1)$$

Developmental time: The developmental time of each mealworm from mean egg hatching date to pupation was recorded in days. The mean date of hatched larvae in all marked boxes was used to define the hatch day for all larvae in one incubator (Appendix 1). There was a weekly check for dead and lost larvae, which were removed (Appendix 2). To calculate the average

developmental time per experimental box (n) the mean was calculated per experimental box. Developmental time of each pupated larvae was aggregated and divided by the number of all pupated larvae.

$$n = \frac{\sum \text{developmental time pupated larvae}}{\sum \text{pupated larvae}} \text{ (in days)} \quad (2)$$

Growth rate: Growth rate of mealworms was calculated from 4th to 6th instar larvae until 95% of larvae pupated per box. Every week the weight of all living larvae per box was measured with a Mettler Toledo MT5 scale. This value was divided by the number of living larvae to receive the average larval weight. The average larval weight per box of week k_1 was subtracted by the average larval weight per box of week k_0 to receive the wet mass increase (of the average larva) in week k_1 (4). The wet mass increase of week k_1 was divided by the average larval weight of week k_0 to receive the growth rate of week k_1. This was multiplied by 100 to receive the growth rate (of the average larva) per box per week in % (5).

The calculation of the mean growth rate per box was weighted by the number of living larvae per week (6) and was calculated until 95 % of pupation per box to reduce the influence of a possible negative growth rate during the pupation time. Negative growth rates can occur naturally when larvae lose weight before they pupate or during their molt period (Urs and Hopkins 1973, Connat et al. 1991 and Ghaly and Alkoaik 2009). And to reduce the influence of a relatively slow developing larva, which was observed in some boxes (Appendix 2). Larvae which did not pupate until 07 June 2019 or which were lost during data collection were dismissed (Appendix 2). The weighted mean growth rates per box (n) were used to compare between incubators and to test the effect of temperature and photoperiod on mealworm growth rate.

$$k_0 = \text{week } 0,\ k_1 = \text{week } 1,\ k_x = \text{last week of data collection},\ gr = \text{growth rate} \quad (3)$$

$$\text{wet mass increase } k_1 = \text{average larva weight } k_1 - \text{average larva weight } k_0 \text{ (in mg)} \quad (4)$$

$$gr\ k_1 = \frac{\text{wet mass increase } k_1}{\text{average larva weight } k_0} * 100 \text{ (in \%)} \quad (5)$$

Weighted mean growth rate:

$$n = \frac{gr\ k_1 * \text{number of living larvae } k_1 + gr\ k_2 * \text{number of living larvae } k_2 + \ldots + gr\ k_x * \text{number of living larvae } k_x}{\text{number of living larvae } k_1 + \text{number of living larvae } k_2 + \ldots + \text{number of living larvae } k_x} \text{ (in \%)} \quad (6)$$

3.5. Genetic analysis

Six *T. molitor* beetles were randomly selected from the stock culture and additional six adult beetles from the natural barn population at WURMFARM were stored for genetic analysis to reveal their genetic structure. The barn population was a natural occurrence of *T. molitor* at the WURMFARM location. The head capsule of each individual was put in a test tube. DNA was extracted with Gentra Puregene kit (QIAGENE) as follows: 100 µl cell lysis and 0,9 µl RNaseA solution were added to test tubes and head capsules were homogenized with a pistil. Protein precipitate (33 µl) was added, test tubes were cold centrifuged at 14,500 g for 4 min. Supernatant with its soluted DNA was put in new tubes and 100 µl isopropanol was added. Samples were mixed and cold centrifuged. Supernatant was discarded and 100 µl ethanol was added to the pellet and samples were cold centrifuged at 14,500 g for 2 min. Supernatant was discarded and tubes were dried overnight. The next day 85 µl hydration solution was added and samples were stored in a freezer. The standard DNA barcoding primers LCO1490 and HCO2198 (Folmer et al. 1994) were used to amplify a 710 bp fragment of mitochondrial cytochrome *c* oxidase subunit I gene (COI). PCR reactions were performed in a 25 µl volume, containing 17.75 µl Milli-Q water, 2.5 µl y-Buffer, 0.5 µl dNTPs, 1 µl of each primer, 0.25 µl *Taq* polymerase and 2 µl DNA sample. The PCR program ran at 94 °C for 3 min, followed by 34 cycles at 94 °C for 30 sec, 55 °C for 45 sec and 72 °C for 45 sec, ending with a final elongation at 72 °C for 5 min. PCR products were stored at 4 °C. Amplified products were tested with 2% agarose gel stained with GelRed Nucleic Acid Dye (Biotum, Hayward, CA, USA) electrophoresis to prove the efficiency of PCR. Subsequently, the samples were sent to the Comprehensive Cancer Center DNA Sequencing & Genotyping Facility of the University of Chicago (USA) to be sequenced by Sanger sequencing method.

Chromas and GeneRunner were used to visualize DNA sequences. Then ClustalX was used to align and compare DNA sequences of each sample. Each sample was blasted online at the National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov). For the Neighbour joining phylogenetic analysis (Saitou and Nei 1987, Tamura et al. 2004) done with MEGA X (Kumar et al. 2018) one *T. molitor* outgroup sequence (KU912382) was included (Rulik and Ahrens s.a.).

4. Results

This thesis tested the influence of temperature and photoperiod on survival rate, developmental time and growth rate of *T. molitor* larvae. Survival rate and growth rate were recorded from 4th to 6th instar larvae until pupation, developmental time from hatch day until pupation. Appendix 2 shows the developmental time of every pupated mealworm and the survival rate and growth rate of every box (n).

4.1. Survival rate

There is a significant effect of temperature ($p = 0.001$, two-way ANOVA, post-hoc: Tukey) on the survival of mealworms (Table 4.1). Across all photoperiods tested, there is a significant difference between the survival rate at 20 °C with a mean value of 92.0% ± 7.0 and the survival rates at 25 °C with 97.0% ± 4.3 ($p = 0.003$) and at 30 °C with 96.7% ± 5.4 ($p = 0.006$), respectively. There is no significant difference between survival rates of the latter two mentioned temperatures ($p = 0.978$) (Table 4.1). The lowest survival rate was recorded at a temperature of 20 °C and 24 D, with a mean survival rate of 90.4% ± 7.6 The highest survival rate was recorded at 25 °C and 24 D, and 30 °C and 24 D with a mean survival rate of 98.5% ± 2.4 and 98.5 ± 3.4, respectively. There is no influence of photoperiod on the survival of mealworms (Table 4.1).

Within 20 °C, 25 °C and 30 °C there was no significant difference between LD, SD and 24 D, each. Within LD, SD and 24 D there are significant difference between temperature regimes at 20 °C and the other two temperature regimes (25 °C and 30 °C) (Table 4.1).

Survival rates (in %) of mealworms at three different temperature regimes are presented in Figure 4.1. The curves show that the differences between 20 °C and the other two regimes already get evident after the first two weeks of data collection.

Table 4.1 Survival rate (in %) of mealworms at 20 °C, 25 °C and 30 °C temperature regimes and LD, SD and darkness D photoperiods. Two-way ANOVA, post hoc: Tukey was performed.
n is the sample size used for statistical analysis.
Individuals is the number of investigated larvae.
† nonsignificant > 0.05, * significant < 0.05 significant, ** significant < 0.01, *** significant < 0.001.
Means followed by the same letter are not significantly different according to LSD 0.05.

Temperature	Photoperiod	Mean ± SD	n	Individuals
20 °C	LD	93.8 ± 5.8^{a}	10	192
	SD	91.9 ± 7.6^{a}	10	199
	24 D	90.4 ± 7.6^{a}	10	196
25 °C	LD	96.5 ± 4.7^{b}	10	200
	SD	96 ± 5.2^{b}	10	200
	24 D	98.5 ± 2.4^{b}	10	199
30 °C	LD	94.1 ± 7.7^{b}	10	196
	SD	97.5 ± 3.6^{b}	10	198
	24 D	98.5 ± 3.4^{b}	10	199
20 °C	Total	**92 ± 7.0****	30	587
25 °C	Total	**97 ± 4.3^{c}**	30	599
30 °C	Total	**96.7 ± 5.4^{c}**	30	593
Total	LD	**94.8 ± 6.1†**	30	588
	SD	**95.1 ± 6†**	30	597
	24 D	**95.8 ± 6.2†**	30	594
	Total	**95.2 ± 6**	90	1779

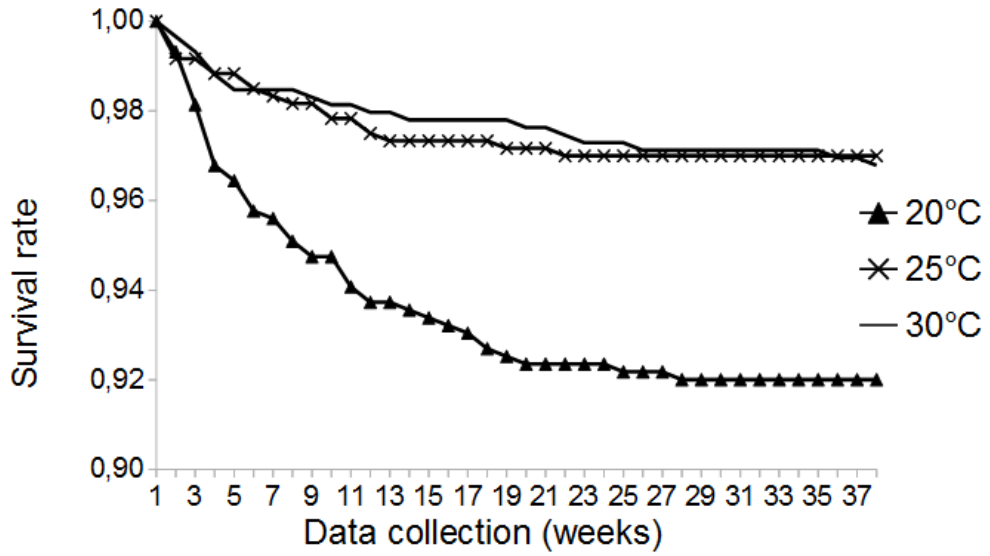

Figure 4.1 Survival rate (in %) of mealworms at three different temperature regimes (20 °C, 25 °C and 30 °C) during data collection (in weeks).

4.2. Developmental time

There is a significant influence of temperature ($p < 0.001$, two-way ANOVA, post-hoc: Tukey) on the larval developmental time, i.e. from larval hatch day until pupation (Table 4.2). Across all photoperiods tested, the developmental time of mealworms at 20 °C with a mean of 184.8 ± 7.9 days is significantly higher than at 25 °C and 30 °C (both $p < 0.001$). There is no significant difference between 25 °C, with a mean developmental time of 138 ± 10.8 days and 30 °C with 136.1 ± 8.7 days ($p = 0.558$) (Table 4.2, Figure 4.2 and 4.3).

There is a significant effect of photoperiod ($p = 0.001$, two-way ANOVA, post-hoc: Tukey) on the developmental time of mealworms. Across all temperatures, the mean developmental time under LD with 156.7 ± 24.1 days is significantly higher than under SD ($p = 0.016$) and 24 D ($p = 0.001$) (Table 4.2, Figure 4.2 and 4.3). There is no significant difference between SD with 151.9 ± 20 days, and 24 D with 150.3 ± 28.7 days ($p = 0.632$) (Table 4.2). Mealworms develop faster under SD or 24 D.

Within 20 °C mealworms developed fastest under SD conditions. The highest overall developmental times occurred at 20 °C and LD with 188.9 ± 4.5 days and 24 D with 189 ± 5.2 days. Within 25 °C they developed fastest under 24 D. Within 30 °C there are no major developmental time differences between different photoperiods. Within LD mealworms developed slower at 20 °C, compared to 25 °C and 30 °C. Within SD there is a gradual decline in developmental time, the higher the temperature. But within 24 D the lowest developmental time 125.6 days ± 4.2 was recorded at 25 °C which was the lowest developmental time recorded in the experiment (Table 4.2, Figure 4.2).

Table 4.2 Developmental time (in days) of mealworms at three temperature regimes (20 °C, 25 °C and 30 °C) and three photoperiods long day LD, short day SD and darkness 24 D (mean ± SD). Two-way ANOVA, post hoc: Tukey was performed.
n in the sample size for statistical analysis.
Individuals is the number of investigated larvae.
† nonsignificant > 0.05, * significant < 0.05 significant, ** significant < 0.01, *** significant < 0.001.
Means followed by the same letter are not significantly different according to LSD 0.05.

Temperature	Photoperiod	Mean ± SD	n	Individuals
20 °C	LD	188.9 ± 4.5^{a}	10	180
	SD	176.4 ± 5.9^{ac}	10	183
	24 D	189 ± 5.2^{ac}	10	177
25 °C	LD	141.5 ± 7.5^{ab}	10	193
	SD	146.8 ± 5.6^{bc}	10	192
	24 D	125.6 ± 4.2^{bc}	10	196
30 °C	LD	139.8 ± 8.3^{ab}	10	185
	SD	132.5 ± 10.1^{bc}	10	193
	24 D	136.3 ± 6.4^{bc}	10	196
20 °C	Total	**184.8 ± 7.9*** **	30	540
25 °C	Total	**138 ± 10.8^{d}**	30	581
30 °C	Total	**136.2 ± 8.7^{d}**	30	574
Total	LD	**156.7 ± 24.1****	30	558
	SD	**151.9 ± 20^{e}**	30	568
	24 D	**150.3 ± 28.7^{e}**	30	569
	Total	**153 ± 24.4**	90	1695

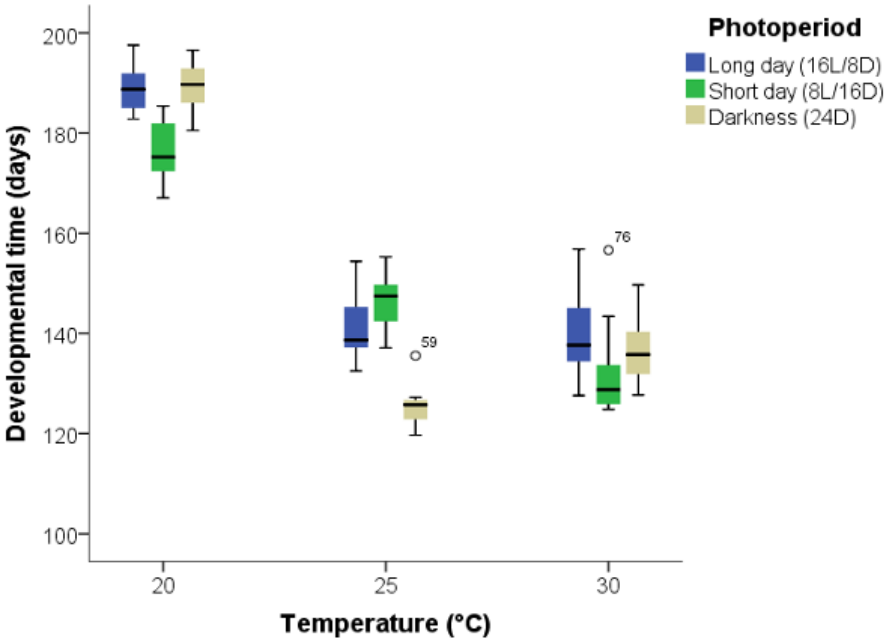

Figure 4.2 Developmental time (in days) at three different temperature regimes (20 °C, 25 °C and 30 °C) grouped after three photoperiods long day LD, short day SD and darkness 24 D.

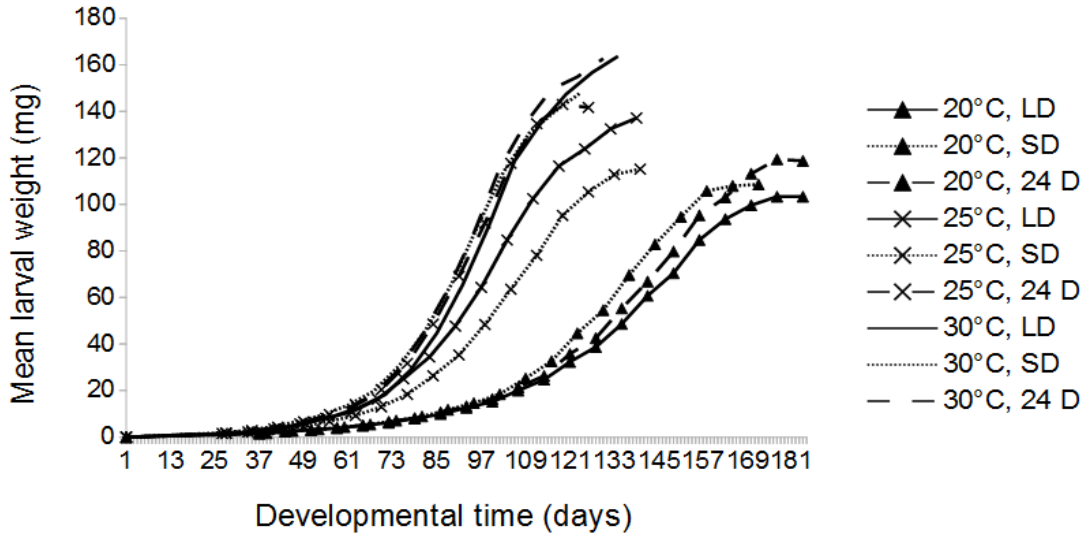

Figure 4.3 Mean larval weight (in mg) during developmental time (in days) of mealworms at 20 °C, 25 °C and 30 °C temperature regimes and LD, SD and 24 D photoperiods until approx. 50% pupation, slopes of curves represent growth rates of all mealworms in one incubator.

4.3. Growth rate

Weekly growth rates of mealworms until 95% pupation at different temperatures and photoperiods showed a significant effect of temperature on growth rate of mealworms ($p < 0.001$, two-way ANOVA, post-hoc: Tukey) (Table 4.3). All three temperature regimes are significantly different (all $p < 0.001$). Across all photoperiods tested, the mean growth rate at 20 °C is the lowest with 25.1% ± 1.8, followed by 25 °C with 36.2% ± 4.5 and 30 °C being the highest with 39.2% ± 3.5 (Table 4.3, Figure 4.3 and 4.4).

There is also a significant effect of photoperiod on the growth rate ($p < 0.001$, two-way ANOVA, post-hoc: Tukey) (Table 4.3). Across all temperatures, the growth rate under 24 D (mean 35.7% ± 8.2) is significantly higher than the growth rate under LD (32.5% ± 7) or SD (32.3% ± 5.1) (both $p < 0.001$). There is no significant difference between growth rates under LD and SD ($p = 0.962$) (Table 4.3). Mealworms at 25 °C or 30 °C gain more weight in the same time under 24 D compared to LD or SD. The highest growth rates were recorded at 25 °C and 30 °C and 24 D, with means of 41.2% ± 1.6 and 41.1% ± 3.7 respectively. The lowest growth rate, 23.7% ± 1.1, occurred at 20 °C and LD (Table 4.3, Figure 4.3 and 4.4).

Within 20 °C the highest mealworm growth rate occurred under SD. Within 25 °C and 30 °C the highest growth rate occurred under 24 D. Within LD and SD there is a gradual increase of growth rates the higher the temperature. Within 24 D there is a difference between 20 °C and the two other temperature regimes, but the mealworm growth rate of 25 °C and 30 °C is similar (Table 4.3, Figure 4.4).

Table 4.3 Growth rate (in %) of mealworms at three temperature regimes (20 °C, 25 °C and 30 °C) and three photoperiods long day LD, short day SD and darkness 24 D (mean ± SD). Two-way ANOVA, post hoc: Tukey was performed.
n is the sample size for statistical analysis.
Individuals is the number of investigated larvae.
† nonsignificant > 0.05, * significant < 0.05 significant, ** significant < 0.01, *** significant < 0.001.
Means followed by the same letter are not significantly different according to LSD 0.05.

Temperature	Photoperiod	Mean ± SD	n	Individuals
20 °C	LD	23.7 ± 1.1^{ad}	10	171
	SD	27 ± 1.1^{ad}	10	177
	24 D	24.8 ± 1.3^{ae}	10	169
25 °C	LD	35.1 ± 3.4^{bd}	10	186
	SD	32.3 ± 2.6^{bd}	10	183
	24 D	41.2 ± 1.6^{be}	10	188
30 °C	LD	38.8 ± 2.7^{cd}	10	177
	SD	37.7 ± 3.5^{cd}	10	184
	24 D	41.1 ± 3.7^{ce}	10	188
20 °C	Total	**25.1 ± 1.8*** **	30	517
25 °C	Total	**36.2 ± 4.5*** **	30	557
30 °C	Total	**39.2 ± 3.5*** **	30	549
Total	LD	**32.5 ± 7^{f}**	30	534
	SD	**32.3 ± 5.1^{f}**	30	544
	24 D	**35.7 ± 8.2*** **	30	545
	Total	**33.5 ± 7**	90	1623

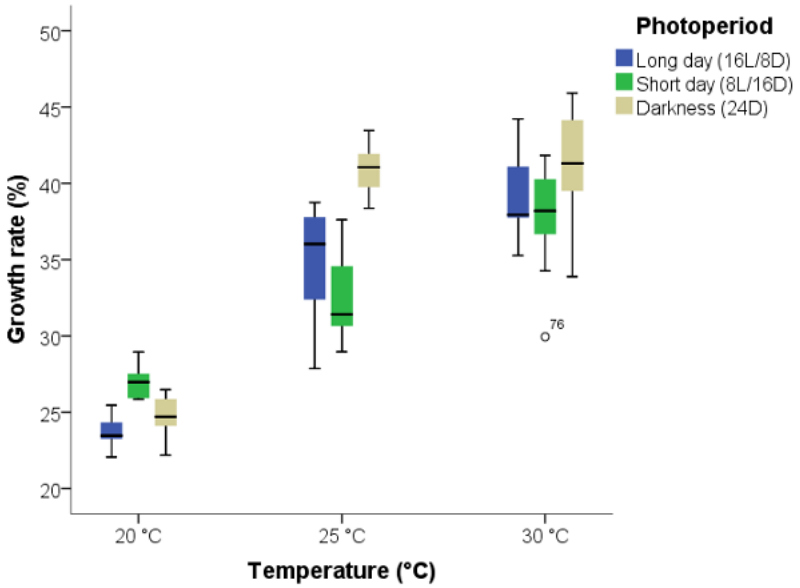

Figure 4.4 Growth rates (in %) of mealworms at three different temperature regimes (20 °C, 25 °C and 30 °C) grouped after three photoperiods long day LD, short day SD and darkness 24 D.

4.4. Genetics

DNA was extracted from six *T. molitor* adults from the stock culture population and six samples from a wild population originating from WURMFARM barn. Sequencing and alignment of the COI sequences showed eight haplotypes, three haplotypes for the stock population and six haplotypes for the wild barn population. Barn population has more haplotypes revealing that the stock population might have undergone a bottleneck. They have one haplotype in common (Tm5, Tm6 and Tm14). One sample from the wild barn population, Tm11, had 12 and therefore most mutations. Other haplotypes had only one to four mutations (Table 4.5, Figure 4.6).

Table 4.4 Eight haplotypes were defined after alignment analyzing six *T. molitor* samples used in this thesis and six *T. molitor* samples from a wild barn population at WURMFARM, Carinthia, Austria. Mutations are listed according to the locations on the COI region between primers LCO1490 and HCO2198.

T. molitor		**Clustalnumber**														
		8	**51**	**59**	**60**	**302**	**326**	**327**	**380**	**437**	**537**	**584**	**587**	**590**	**596**	**626**
Thesis pop.	1	A	C	A	G	A	T	G	G	C	T	A	G	G	A	T
	2	A	C	A	G	A	T	G	G	C	T	A	G	G	A	T
	3	A	C	A	G	A	T	G	G	C	T	A	G	G	A	T
	4	A	C	A	G	C	T	A	G	T	T	A	A	G	A	T
	5	A	C	A	G	A	T	G	G	C	T	A	A	G	A	T
	6	A	C	A	G	A	T	G	G	C	T	A	A	G	A	T
Barn pop.	11	T	T	G	G	G	C	G	A	C	C	G	A	A	G	G
	12	A	C	A	G	A	T	G	G	C	T	A	A	G	A	G
	13	A	C	A	A	A	T	G	G	C	T	A	A	G	A	G
	14	A	C	A	G	A	T	G	G	C	T	A	A	G	A	T
	15	A	C	A	G	G	T	G	G	C	T	A	G	G	A	T
	16	A	C	A	G	G	T	G	G	C	T	A	G	G	A	G

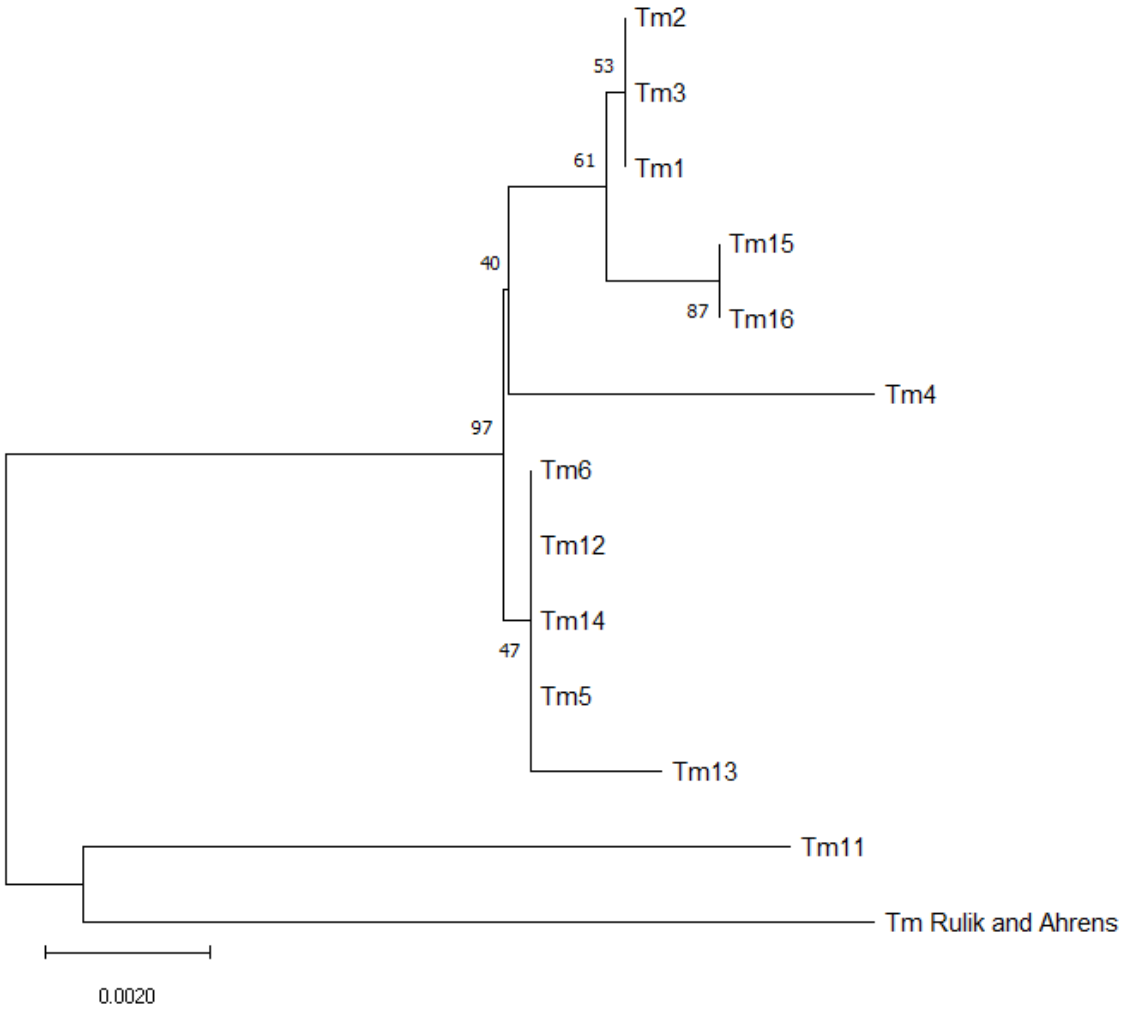

Figure 4.5 Evolutionary relationships of taxa, using the Neighbor-Joining method (Saitou and Nei 1987, Tamura et al. 2004), the optimal tree with the sum of branch length = 0.03445508 is shown, the percentage of replicate trees which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). This analysis involved thirteen nucleotide sequences, Tm1-6 samples from a population used in this thesis, Tm11-16 samples from wild barn population and as kind of outgroup species a *T. molitor* sequence from the Genbank was taken (KU912382) (Rulik and Ahrens s.a.). Codon positions included were 1^{st}+2^{nd}+3^{rd}+Noncoding, all ambiguous positions were removed for each sequence pair (pairwise deletion option), there were a total of 625 positions in the final dataset, conducted in MEGA X (Kumar et al. 2018).

5. Discussion

5.1. Survival rate

There was a significant influence of temperature on survival rates of mealworms, but no significant influence of photoperiod on survival. Mealworms died more frequently at 20 °C, with a mean survival rate of 92% ± 7 compared to the two other temperature regimes with a survival rate of about 97%.

More mealworms died at the beginning of the experiments. The longer the experiment the more mealworms pupated. Punzo and Mutchmor (1980) describe that young larvae are more susceptible to temperature extremes, 10 °C and 35 °C, than old larvae, however, with no difference in mortality among larval instars at 25°C. These differences between larval age were not found in this study, as data collection began in instar four to six with a larval age of about four to five weeks. Therefore, the relative high survival rates in this experiment could be lower at 20 °C if data collection started with larval hatch.

Weaver and McFarlane (1990) tested the survival of mealworms at different densities at 30 °C. After one month of development larvae had a mean survival rate of 97%. This is very similar to the survival rate presented in this thesis. At 25 °C Greenberg and Ar (1996) recorded a larval survival rate of 96% from hatch day until pupation. Punzo and Mutchmor (1980) recorded a survival rate of 100% at 25 °C and Huang et al. (2011) obtained an overall survival rate of 92% for the first 150 days of larval development at 25-30 °C. All four studies used wheat bran as feed, unfavorable for a fast development (Oonincx et al. 2015, van Broekhoven et al. 2015), but they balanced a negative effect by suppling a water source (lettuce, apples or carrots).

van Broekhoven et al. (2015) recorded a survival rate of 86% ± 9.6 from hatch day until pupation at 28 °C. Despite a similar diet compared to this thesis experiment, the survival rate is about 10% lower than the survival rate at 25 °C or 30 °C analysed here. A possible reason might be that van Broekhoven et al. (2015) used of first instar larvae and therefore a higher mortality of young instars was observed. They recorded higher survival rates of about 90% with diets high in protein (van Broekhoven et al. 2015).

Other studies recorded lower survival rates during mealworm development of 70-84% at temperatures of 26-28 °C (Urs and Hopkins 1973, Oonincx et al. 2015, Dreassi et al. 2017).

Differences in the survival rates at 25°C and 30°C in this thesis originate mainly from different starting points of data collection and from different feeds.

In general results from my experiments confirm previous studies. The higher the temperature, the higher the survival rate of mealworms with an optimal farming temperature between 25 °C and 30 °C. Also, Punzo and Mutchmor (1980) and Koo et al. (2013) state that the temperature preference of *T. molitor* is between 22-28°C and that temperatures above 35°C and below 20°C are associated with decreasing survival rates and stress. Other important factors influencing mealworm survival are water source, feed and cannibalism (at high densities) (Tschinkel and Willson 1971, Punzo and Mutchmor 1980, Weaver and McFarlane 1990, Morales-Ramos et al. 2010, Oonincx et al. 2015, van Broekhoven et al. 2015, Kim et al. 2017, Zhang et al. 2019, Rumbos et al. 2020).

Future research should test survival rates at additional temperature regimes, especially between 20 °C and 35 °C, as there could be higher survival rates between temperature regimes tested in this thesis.

A comparison between *Alphitobius diaperinus* and *T. molitor* is suitable because both belong to the family of Tenebrionidae and both are farmed for human consumption (van Broekhoven et al. 2015, Bjorge et al. 2018). Rueda and Axtell (1996) found a similar significant influence of temperature on survival of *A. diaperinus* larvae, as compared to the *T. molitor* larvae in this thesis. *Alphitobius diaperinus* survival rate at 20 °C was significantly lower than survival rates at 25 °C and 30 °C. At 25 °C and 30 °C larvae of *A. diaperinus* and *T. molitor* have similar survival rates (Urs and Hopkins 1973, Rueada and Axtell 1996, Oonincx et al. 2015, Dreassi et al. 2017). Rueada and Axtell (1996) also tested *A. diaperinus* survival rates at 35 °C and 38 °C, which were not significantly different to 25 °C and 30 °C. This is in contrast to a mealworm survival rate at 35 °C which is significantly lower than at 25 °C (Punzo and Mutchmor 1980). This confirms a trend that *A. diaperinus* is more heat-resistant than *T. molitor* (Kim et al. 2017b, Bjorge et al. 2018).

There are many studies which found an effect of photoperiod on the development of insects. Kutcherov et al. (2018) tested the influence of temperature and photoperiod on survival of *Scantius aegyptius* and *Timarcha tenebricosa*. Larvae of *S. aegyptius* had higher survival rates, the higher the temperature. At 26 °C and 28 °C and LD (16 L:8 D) survival was significantly higher compared to 10 L:14 D photoperiod. Larvae of *T. tenebricosa* had a significantly higher mortality at 26 °C, compared to lower temperatures. The influence of photoperiod was similar

with a significantly higher survival rate under LD photoperiod compared to a 12 L:12 D photoperiod (Reference).

Savvidou and Bell (1994) found no significant influence of photoperiod on survival of *Gnatocerus cornutus* (Coleoptera: Tenebrionidae) larvae. They tested 12 L:12 D, 15 L:9 D, 24 L and 24 D photoperiods.

5.2. Developmental time

There is a significant influence of temperature and photoperiod on developmental times of mealworms. Generally, results show that the higher the temperature, the lower the developmental times with no significant difference between 25 °C and 30 °C. The lowest developmental time 125.6 ± 4.2 days was recorded at 25 °C and 24 D. Rearing under LD resulted in significantly longer developmental times, than under SD and 24 D.

At all three temperature regimes developmental times fit well with literature findings. Koo et al. (2013) found significant differences between the developmental time at 20 °C (200 days) and 25 °C (127 days) and 30 °C (111 days). The difference between 20 °C and 25 °C was relatively greater than the difference between 25 °C and 30 °C, which is in accordance to this thesis. The difference in significance between 25 °C and 30 °C can result from a fluctuating relative humidity of experiments by Koo et al. (2013), which is specified with 60-70% and indicated with a high standard deviation. However, the general trend of a faster development at higher temperatures is confirmed, which can be explained by the van't Hoff equation, as insect are poikilothermic (Mortimer and Müller 2007).

Ludwig and Fiore (1960) found the lowest developmental times at 25 °C (115.5-134.8 days) and considerable higher developmental times at 30 °C (141.4-163.6 days). They did not test if these differences are significant. Their results are similar to this thesis results at 25 °C (125.6-146.8 days) and are slightly higher compared to this thesis results at 30 °C (132.5-139.8 °C). Differences between 25 °C and 30 °C are greater than the non-significant difference at the same temperature regimes from this thesis study for unknown reasons.

There is a great range of developmental time of mealworms in literature, which is mainly due to different feeds and availability of a water source. Lower values (76-87.7 days) at 25 °C and 30 °C originate from studies with favorable feed and a water source (Urs and Hopkins 1973,

Morales-Ramos et al. 2010, Oonincx et al. 2015, van Broekhoven et al. 2015). Upper values (202.5-227 days) originate from studies with unfavorable feed and no water source (Urs and Hopkins 1973, Oonincx et al. 2015). Developmental times at 25 °C and 30 °C in this study are in the mid-value range of literature data and similar to findings of Huang et al. (2011) and Morales-Ramos et al. (2015). This disparity can be due to a lack of a water source, which could have been compensated by a higher relative humidity or a water source e.g. carrots. Feed and water availability besides temperature, are important conditions influencing the developmental time of mealworms (Ludwig and Fiore 1960, Urs and Hopkins 1973, Morales-Ramos et al. 2010, Oonincx et al. 2015, van Broekhoven et al. 2015, Kim et al. 2017). Further, differences can originate from the definition of developmental time. van Broekhoven et al. (2015) for example defined the developmental time from hatch day until 50% of experimental larvae pupated. This thesis developmental time was defined from hatch day until pupation.

The influence of photoperiod on developmental time of mealworms in this thesis experiment is not that distinct. This study found a significantly higher developmental time under long-day conditions compared to other photoperiods. If tested just at 25 °C and 30 °C, developmental time under 24 D was significantly lower, than under LD or SD. Either way the differences in developmental time between different photoperiods is low, between five and ten days with a higher standard deviation, compared to the difference between 20 °C and higher temperatures, being more than 45 days. This leads to the assumption that the influence of photoperiod seems to be low, compared to other major factors like temperature, water source and feed.

Cloudsley-Thompson (1953) states that a diurnal locomotory rhythm occurs in some of the observed mealworms. Their normal diurnal response is to avoid daylight by digging into the substrate and be more active at night (Cloudsley-Thompson 1953, Punzo 1975). They can become arrhythmic in their diurnal locomotory rhythm but there is no statement if this results in overall more activity at 24 D and therefore a faster development, as depicted by results in this thesis (Cloudsley-Thompson 1953).

Ribeiro et al. (2018) state, that photoperiod influences development of mealworms, but do not specify the effect (Tyshchenko and Ba 1986 cited Ribeiro et al. 2018).

Kim et al. (2015) found a significantly lower developmental time at 25 °C under 14 L:10 D photoperiod (157.35 days), compared to 12 L:12 D (184.89 days) and 10 L:14 D (179.56 days). This contradicts findings in this thesis as there were lower developmental times at 25 °C under 24 D (125.6 days), compared to LD (141.5 days) and SD (146.8 days). Kutcherov et al. (2018)

describes that some insects develop faster at LD compared to SD to complete an additional generation under favorable summer conditions. Others, e.g. spring active species, try to avoid unfavorable conditions (e.g. hot temperatures in summer) as a larva and therefore also develop faster at LD compared to SD (Kutcherov et al. 2018). Mealworms develop faster at LD (Kim et al. 2015) for similar reasons but there is no evidence to back this hypothesis.

According to the results from this study and literature the optimal farming temperature for a fast mealworm development is at 30 °C and the optimal photoperiod needs further research, especially under farming conditions. Research should also focus on the interaction of temperature and photoperiod and the effect on mealworm development, as photoperiod can modify the thermal reaction norm (Lopatina et al. 2011).

Rueda and Axtell (1996) and Kim et al. (2017b) recorded significantly lower developmental times for *A. diaperinus* larvae, the higher the temperature, which follows the same trend as the developmental response to temperature of mealworms. At 35 °C the developmental time for *A. diaperinus* larvae was even lower, which is a further indicator for the more heat-adapted *A. diaperinus* compared to *T. molitor*, which is exposed to stress at 35 °C (Punzo and Mutchmor 1980). Larvae of *A. diaperinus* develop faster than mealworms. This is due to their smaller size, weight, less larval instars and faster life cycle at optimal conditions (Wilson and Miner 1969, Rueda and Axtell 1996, Morales-Ramos et al. 2010, van Broekhoven et al. 2015 and Kim et al. 2017b).

Wang et al. 2013 recorded significantly slower developmental times under 24 D, than under SD or LD for *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae) larvae, but Reznik et al. (2015), recorded significantly slower developmental times under LD compared to SD for *Harmonia axyridis* larvae from the same taxonomic family. Savvidou and Bell (1994) found no significant influence of photoperiod on developmental time from larvae to adult emergence for *Gnatocerus cornutus* (Coleoptera: Tenebrionidae).

5.3. Growth rate

There is a significant influence of temperature and photoperiod on growth rate of mealworms. Generally, results show that the higher the temperature, the higher the growth rates. Rearing under 24 D resulted in a significantly higher growth rate, than under LD and SD light conditions.

Weekly growth rates of mealworms are similar to literature findings. Kim et al. (2016) and van Broekhoven et al. (2015) recorded larval weights at 25 °C and 28 °C, respectively. Their weekly growth rates (40.1-49%), calculated by the author here, of mealworms fed with similar feed are higher than weekly growth rates in this thesis. This is likely due to their additional feeding of cabbage leaves and carrots, respectively, as water source (van Broekhoven et al. 2015, Kim et al. 2016). In case of van Broekhoven et al. (2015) the growth rate was calculated from data, which was recorded from four-week old larvae until the first pupa was observed. Starting point of data collection was similar to this thesis, but a recording until the first pupation leads to a higher growth rate compared to a recording until 95% pupation, as conducted in this thesis, because during last instars and before pupation mealworms have a lower growth rate, than before (Urs and Hopkins 1973, Ghaly and Alkoaik 2009, Morales-Ramos and Rojas 2015, Bjorge et al. 2018).

Urs and Hopkins (1973) found a daily growth rate of 4-7% at 26.7 °C over the entire larval stage, which is in accordance to a daily growth rate of approximately 5.2% at 25 °C in this thesis.

Bjorge et al. (2018) recorded daily growth rates of mealworms at 18.7 °C, 25.4 °C and 31 °C between 25-75% during their developmental time. They found a significantly higher growth rate, the higher the temperature with a peak of 16.7% daily growth rate at 31 °C, which represents the same peak and differences in significance between the three temperature regimes used in this thesis. There are remarkable differences especially at 25 °C and 30 °C, where Bjorge et al. (2018) recorded twice as high daily growth rates (12% and 16.7%), compared to daily growth rates in this thesis (6.3% and 7.1%). This can be explained by their experimental feed, which is developed for a high growth rate in *T. molitor* and additional provision of *ad libitum* carrots as water source (Bjorge et al. 2018).

Similar to developmental time, temperature, feed and water source are among the most important conditions to enable a fast weight gain in a short time, hence a high growth rate (Fraenkel et al. 1950 cited in Machin 1975, Ludwig and Fiore 1960, Murray 1968, Urs and Hopkins 1973, Morales-Ramos et al. 2010, Oonincx et al. 2015, van Broekhoven et al. 2015, Kim et al. 2016, Dreassi et al. 2017, Kim et al. 2017, Bjorge et al. 2018, Mancini et al. 2019, Liu et al. 2020, Rumbos et al. 2020).

The influence of photoperiod on the growth rate of mealworms is relatively low with a higher growth rate under darkness, than under light conditions. The difference is significant but just about 3.5% (see Chapter 4.3). Ribeiro et al. (2018) state, that photoperiod influences growth of mealworms, but do not specify the effect (Tyshchenko and Ba 1986 cited Ribeiro et al. 2018). Further research is needed, especially under constant farming conditions.

Bjorge et al. (2018) also calculated the growth rate of *A. diaperinus* larvae. The highest daily growth rate of 18.3% was also recorded at 31 °C which is similar to the growth rate of mealworms in their publication. *A. diapernius* larvae grow slower at lower temperatures and faster at higher temperatures, compared to mealworms. This indicates that *A. diaperinus* is more heat adapted, than *T. molitor* (Bjorge et al. 2018).

An additional example shows, that larval growth rate of the carabid beetle *Amara communis* is also influenced by photoperiod. At 22 °C and 12 L:12 D photoperiod the growth rate of 27% is significantly higher, than under a 22 L:2 D photoperiod with a growth rate of 20% (Lopatina et al. 2011). At this temperature regime larval developmental time is significantly influenced by photoperiod but larval weight is not. Hence this beetle developed faster under 12 L:12 D compared to LD but had a similar end weight. Thus, the growth rate was higher at 12 L:12 D, compared to LD. This is an example of the correlation between developmental time, weight gain and growth rate (Lopatina et al. 2011). Hence this correlation is important for a mealworm production and factors which influence developmental time, also influence growth rates.

The calculation of growth rate, as wet mass increase divided by former wet mass, as implemented in this study, is a simple calculation with limited significance for a commercial mealworm production. There are more sophisticated calculations expressing the growth and which are used to increase effectiveness in production facilities. Waldbauer (1968) defined the food conversion efficiency (FCE) as weight gain of an animal per weight of eaten food. For insects, efficiency of ingested food (ECI) and efficiency of digested food (ECD) is used, which is weight gained per weight of ingested (digested respectively) food on a dry matter basis * 100

(Waldbauer 1968). These calculations incorporate the amount of feed eaten (or even digested) by animals and are therefore superior compared to the growth rate calculated in this thesis, because a production facility can save feed at the optimal FCE/ECI/ECD and therefore produce more cost effective. There are several studies which reveal the optimal FCE/ECI/ECD for mealworms fed with different feed (Morales-Ramos et al. 2011, Oonincx et al. 2015, van Broekhoven et al. 2015, Mancini et al. 2019, Melis et al. 2019, Zhang et al. 2019, Rumbos et al. 2020). But there are no studies which use these calculations to research the optimal temperature or photoperiod for mealworm rearing.

Bjorge et al. (2018) calculated the energy conversion efficiency which is the energy assimilated divided by the energy turnover for mealworms at different temperatures. In other words, it is the ratio of energy which is incorporated into the biomass to the energy which was used for metabolism. For mealworms it is higher at 23.3 °C than at 31 °C. This means at 23.3 °C the conversion rate of feed energy into biomass is higher than at 31 °C and indicates are more cost effective production of mealworms at 23.3 °C (Bjorge et al. 2018).

All in all, the highest growth rate of mealworms occurs at 30 °C and under darkness but depending on the farming objective and calculation method it could be more cost effective to farm mealworms at lower temperatures.

5.4. Genetic analysis

Tenebrio molitor is a synanthropic species, which infests stored products (e.g. granaries). Therefore, several populations are living in environments with constant temperature and light conditions or are being farmed at constant conditions. This leads to the general question whether these populations already adapted to, e.g. constant darkness, and therefore have a different developmental response to a specific photoperiod, compared to a population living in a natural habitat. The origin and habitat conditions of experimental mealworms is therefore of great importance. Six samples from the stock population used in this experiment have three haplotypes. Urs and Hopkins (1973) found significantly different developmental times between two strains of mealworms. This might indicate a genetic influence on development of mealworms (Urs and Hopkins 1973). Huang et al. (2011) found different developmental characteristics between yellow and black colored varieties of *T. molitor* larvae. There might be a genetic difference within one great population used for farming purposes and therefore

possible differences in development and potential for artificial selection towards an improved biomass productivity could be assumed (Morales-Ramos et al. 2019). How mealworm farmers can utilize this remains a question of future research.

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7. List of figures

8. List of tables

Appendix 1

Determination of larval hatch day and end of data collection.

Table A1

Dates of procedure and determination of larval hatch day for each incubator by checking marked boxes daily and calculating the mean.

Incubator	I1	I2	I3	I4	I5	I6	I7	I8	I9
Temperature (°C)	20	20	20	25	25	25	30	30	30
Photoperiod	16L/8D	8L/16D	24D	16L/8D	8L/16D	24D	16L/8D	8L/16D	24D
Filling incubator with beetles	17.07.2018	18.07.2018	18.07.2018	18.07.2018	18.07.2018	19.07.2018	19.07.2018	19.07.2018	24.07.2018
First larva sighting marked box 1	08.08.2018	06.08.2018	08.08.2018	01.08.2018	28.07.2018	28.07.2018	26.07.2018	30.07.2018	30.07.2018
First larva sighting marked box 2	04.08.2018	06.08.2018	07.08.2018	31.07.2018	28.07.2018	30.07.2018	27.07.2018	27.07.2018	30.07.2018
First larva sighting marked box 3	no larvae found	03.08.2018	no larvae found	no eggs found	30.07.2018	30.07.2018	27.07.2018	30.07.2018	30.07.2018
First larva sighting marked box 4	no larvae found	06.08.2018	no larvae found	28.07.2018	28.07.2018	28.07.2018	26.07.2018	27.07.2018	30.07.2018
First larva sighting marked box 5	12.08.2018	09.08.2018	no larvae found	31.07.2018	28.07.2018	30.07.2018	25.07.2018	30.07.2018	no larvae found
Mean larval hatch day	**08.08.2018**	**06.08.2018**	**08.08.2018**	**30.07.2018**	**29.07.2018**	**29.07.2018**	**26.07.2018**	**29.07.2018**	**30.07.2018**
End of data collection	**04.06.2019[a]**	**07.06.2019[a]**	**07.06.2019[a]**	**30.03.2019[b]**	**10.03.2019[b]**	**07.06.2019[a]**	**07.06.2019[a]**	**07.06.2019[a]**	**07.06.2019[a]**

[a]still at least one living larvae

[b]last pupa

Appendix 2

Detailed depiction of survival rate, developmental time, from hatch day until pupation, and growth rate, until 95% pupation, per box (Table A2), developmental time, from hatch day until pupation, and pupation day or day of death of every larva (Table A3-A11) and growth rate, until 95% pupation, of every box (Table A12-A20). Calculation method depicted in chapter 3.4..

Table A2

Survival rate (Sr) in %, average developmental time (Dt) in days and weighted mean growth rate (Gr) in % per box.

Incubator	Condition	Box (n)	Sr (%)	Dt (days)	Gr (%)	Incubator	Condition	Box (n)	Sr (%)	Dt (days)	Gr (%)
I1	20°C, 16L/8D	1	89.5	191.3	24.3	I6	25 °C, 24D	51	100	126.4	39.8
		2	85	183.5	25.5			52	95	122.1	43.5
		3	95	185.1	25.3			53	95	125.6	41.8
		4	94.7	188.8	23.4			54	100	126.7	38.4
		5	100	188.7	23.4			55	100	122.9	41.2
		6	84.2	192.3	23.8			56	100	119.7	43.4
		7	100	182.8	23.3			57	100	125.9	39.7
		8	94.7	197.6	22.1			58	100	123.9	41.9
		9	94.7	191.9	22.1			59	100	135.6	41.0
		10	100	187.1	23.5			60	95	127.3	41.0
I2	20°C, 8L/16D	11	95	172.6	27.1	I7	30 °C, 16L/8D	61	95	134.2	37.9
		12	95	173.1	28.3			62	95	146.7	36.1
		13	75	177.4	29.0			63	95	134.9	38.4
		14	90	172.4	26.9			64	100	135.8	41.1
		15	100	182.0	25.9			65	100	143.1	37.8
		16	90	172.1	27.5			66	100	139.5	38.0
		17	95	178.2	25.9			67	76.5	145.1	37.8
		18	95	167.1	27.4			68	100	127.6	41.2
		19	100	185.4	25.9			69	95	134.4	44.2
		20	84.2	183.6	26.2			70	85	156.8	35.3
I3	20°C, 24D	21	94.7	186.1	26.5	I8	30 °C, 8L/16D	71	100	125.9	40.5
		22	94.7	196.6	24.1			72	94.7	143.4	34.3
		23	80	187.0	24.3			73	100	126.6	40.3
		24	90	189.8	25.9			74	95	133.7	38.6
		25	90	191.1	23.7			75	90	127.3	36.7
		26	75	194.6	24.2			76	100	156.6	30.0
		27	95	189.5	25.2			77	100	130.3	37.3
		28	89.5	192.9	22.2			78	95	124.8	41.8
		29	95	182.0	26.2			79	100	125.4	39.8
		30	100	180.5	25.5			80	100	130.7	37.9
I4	25 °C, 16L/8D	31	90	139.2	36.1	I9	30 °C, 24D	81	90	149.7	33.9
		32	90	138.2	35.8			82	100	138.5	45.0
		33	90	133.9	37.8			83	100	140.8	40.9
		34	100	144.1	32.4			84	100	127.7	44.1
		35	95	137.5	36.4			85	100	131.9	39.5
		36	100	154.4	27.9			86	100	133.1	41.7
		37	100	132.5	37.9			87	100	140.4	37.2
		38	100	145.3	35.9			88	100	135.5	42.6
		39	100	153.0	31.8			89	100	136.1	39.8
		40	100	137.2	38.8			90	95	129.3	45.9
I5	25 °C, 8L/16D	41	100	142.4	34.6						

	42	90	140.4	34.6
	43	95	152.7	30.6
	44	100	137.2	37.6
	45	95	155.3	29.0
	46	100	145.8	31.6
	47	100	148.4	31.2
	48	85	149.6	30.7
	49	100	149.8	31.2
	50	95	146.5	32.0

Table A3
Incubator 1 (I1), 20°C, 16L/8D, mean hatch day 08.08.2018, developmental time (Dt) in days and pupation day or day of death (Dead larvae) of every larva.

Box (n)	Larva	Pupation day	Dt (days)	Dead larvae	Box (n)	Larva	Pupation day	Dt (days)	Dead larvae
1	1	5.2.19	181		6	1	5.2.19	181	
	2	5.2.19	181			2	5.2.19	181	
	3	12.2.19	188			3	5.2.19	181	
	4	12.2.19	188			4	5.2.19	181	
	5	12.2.19	188			5	5.2.19	181	
	6	12.2.19	188			6	12.2.19	188	
	7	12.2.19	188			7	12.2.19	188	
	8	12.2.19	188			8	12.2.19	188	
	9	12.2.19	188			9	19.2.19	195	
	10	19.2.19	195			10	19.2.19	195	
	11	19.2.19	195			11	19.2.19	195	
	12	19.2.19	195			12	19.2.19	195	
	13	19.2.19	195			13	26.2.19	202	
	14	19.2.19	195			14	26.2.19	202	
	15	19.2.19	195			15	5.3.19	209	
	16	26.2.19	202			16	10.3.19	214	
	17	26.2.19	202			17			5.3.19
	18			15.1.19		18			22.1.19
	19			24.9.18		19			15.1.19
	20	Larva lost				20	Alive instar 16		
2	1	22.1.19	167		7	1	22.1.19	167	
	2	29.1.19	174			2	29.1.19	174	
	3	29.1.19	174			3	29.1.19	174	
	4	29.1.19	174			4	29.1.19	174	
	5	5.2.19	181			5	29.1.19	174	
	6	5.2.19	181			6	29.1.19	174	
	7	5.2.19	181			7	29.1.19	174	
	8	5.2.19	181			8	29.1.19	174	
	9	12.2.19	188			9	5.2.19	181	
	10	12.2.19	188			10	5.2.19	181	
	11	12.2.19	188			11	5.2.19	181	
	12	12.2.19	188			12	5.2.19	181	
	13	12.2.19	188			13	5.2.19	181	
	14	12.2.19	188			14	12.2.19	188	
	15	12.2.19	188			15	19.2.19	195	
	16	12.2.19	188			16	19.2.19	195	
	17	26.2.19	202			17	19.2.19	195	
	18			25.12.18		18	26.2.19	202	

	19			18.12.18		19	5.3.19	209	
	20			9.10.18		20	Alive instar 11		
3	1	29.1.19	174		8	1	23.1.19	168	
	2	29.1.19	174			2	30.1.19	175	
	3	29.1.19	174			3	30.1.19	175	
	4	5.2.19	181			4	6.2.19	182	
	5	5.2.19	181			5	6.2.19	182	
	6	5.2.19	181			6	13.2.19	189	
	7	5.2.19	181			7	13.2.19	189	
	8	5.2.19	181			8	20.2.19	196	
	9	5.2.19	181			9	20.2.19	196	
	10	5.2.19	181			10	20.2.19	196	
	11	5.2.19	181			11	20.2.19	196	
	12	5.2.19	181			12	27.2.19	203	
	13	12.2.19	188			13	27.2.19	203	
	14	12.2.19	188			14	6.3.19	210	
	15	12.2.19	188			15	6.3.19	210	
	16	12.2.19	188			16	20.3.19	224	
	17	12.2.19	188			17	27.3.19	231	
	18	19.2.19	195			18	27.3.19	231	
	19	26.3.19	230			19			27.3.19
	20			6.11.18		20	Alive instar 14		
4	1	15.1.19	160		9	1	23.1.19	168	
	2	22.1.19	167			2	23.1.19	168	
	3	29.1.19	174			3	30.1.19	175	
	4	29.1.19	174			4	30.1.19	175	
	5	29.1.19	174			5	6.2.19	182	
	6	29.1.19	174			6	6.2.19	182	
	7	5.2.19	181			7	13.2.19	189	
	8	5.2.19	181			8	13.2.19	189	
	9	5.2.19	181			9	20.2.19	196	
	10	5.2.19	181			10	20.2.19	196	
	11	5.2.19	181			11	20.2.19	196	
	12	12.2.19	188			12	27.2.19	203	
	13	12.2.19	188			13	27.2.19	203	
	14	19.2.19	195			14	27.2.19	203	
	15	19.2.19	195			15	27.2.19	203	
	16	26.2.19	202			16	27.2.19	203	
	17	26.2.19	202			17	6.3.19	210	
	18	4.6.19	300			18	10.3.19	214	
	19			4.12.18		19			26.9.18
	20	Alive instar 16				20	Alive instar 15		
5	1	22.1.19	167		10	1	23.1.19	168	
	2	22.1.19	167			2	23.1.19	168	
	3	29.1.19	174			3	23.1.19	168	
	4	29.1.19	174			4	30.1.19	175	
	5	29.1.19	174			5	30.1.19	175	
	6	5.2.19	181			6	30.1.19	175	
	7	5.2.19	181			7	30.1.19	175	
	8	5.2.19	181			8	6.2.19	182	
	9	5.2.19	181			9	6.2.19	182	
	10	5.2.19	181			10	6.2.19	182	
	11	12.2.19	188			11	6.2.19	182	
	12	12.2.19	188			12	13.2.19	189	
	13	12.2.19	188			13	13.2.19	189	
	14	19.2.19	195			14	13.2.19	189	
	15	26.2.19	202			15	20.2.19	196	
	16	26.2.19	202			16	20.2.19	196	
	17	10.3.19	214			17	6.3.19	210	

	18	20.3.19	224			18	20.3.19	224	
	19	20.3.19	224			19	26.3.19	230	
	20	Alive instar 16				20	Alive instar 16		

Table A4
Incubator 2 (I2), 20°C, 8L/16D, mean hatch day 06.08.2018, developmental time (Dt) in days and pupation day or day of death (Dead larvae) of every larva.

Box (n)	Larva	Pupation day	Dt (days)	Dead larvae	Box (n)	Larva	Pupation day	Dt (days)	Dead larvae
11	1	1.1.19	148		16	1	8.1.19	155	
	2	1.1.19	148			2	8.1.19	155	
	3	15.1.19	162			3	15.1.19	162	
	4	15.1.19	162			4	15.1.19	162	
	5	15.1.19	162			5	15.1.19	162	
	6	15.1.19	162			6	22.1.19	169	
	7	15.1.19	162			7	22.1.19	169	
	8	22.1.19	169			8	22.1.19	169	
	9	22.1.19	169			9	29.1.19	176	
	10	22.1.19	169			10	29.1.19	176	
	11	22.1.19	169			11	29.1.19	176	
	12	29.1.19	176			12	29.1.19	176	
	13	29.1.19	176			13	29.1.19	176	
	14	29.1.19	176			14	29.1.19	176	
	15	5.2.19	183			15	29.1.19	176	
	16	5.2.19	183			16	5.2.19	183	
	17	12.2.19	190			17	12.2.19	190	
	18	19.2.19	197			18	12.2.19	190	
	19	10.3.19	216			19			27.11.18
	20			27.11.2018		20			1.10.18
12	1	8.1.19	155		17	1	15.1.19	162	
	2	15.1.19	162			2	15.1.19	162	
	3	15.1.19	162			3	22.1.19	169	
	4	22.1.19	169			4	22.1.19	169	
	5	22.1.19	169			5	22.1.19	169	
	6	22.1.19	169			6	22.1.19	169	
	7	22.1.19	169			7	29.1.19	176	
	8	22.1.19	169			8	29.1.19	176	
	9	22.1.19	169			9	29.1.19	176	
	10	22.1.19	169			10	29.1.19	176	
	11	29.1.19	176			11	29.1.19	176	
	12	29.1.19	176			12	5.2.19	183	
	13	29.1.19	176			13	5.2.19	183	
	14	29.1.19	176			14	5.2.19	183	
	15	29.1.19	176			15	12.2.19	190	
	16	29.1.19	176			16	12.2.19	190	
	17	5.2.19	183			17	12.2.19	190	
	18	12.2.19	190			18	12.2.19	190	
	19	19.2.19	197			19	19.2.19	197	
	20			1.10.18		20			25.9.19
13	1	15.1.19	162		18	1	2.1.19	149	
	2	22.1.19	169			2	9.1.19	156	
	3	22.1.19	169			3	9.1.19	156	
	4	22.1.19	169			4	9.1.19	156	
	5	29.1.19	176			5	9.1.19	156	

	6	29.1.19	176			6	9.1.19	156	
	7	29.1.19	176			7	16.1.19	163	
	8	29.1.19	176			8	16.1.19	163	
	9	29.1.19	176			9	16.1.19	163	
	10	29.1.19	176			10	23.1.19	170	
	11	5.2.19	183			11	23.1.19	170	
	12	5.2.19	183			12	23.1.19	170	
	13	12.2.19	190			13	30.1.19	177	
	14	12.2.19	190			14	30.1.19	177	
	15	12.2.19	190			15	30.1.19	177	
	16			6.11.18		16	30.1.19	177	
	17			22.10.18		17	30.1.19	177	
	18			9.10.18		18	30.1.19	177	
	19			1.10.18		19	6.2.19	184	
	20			1.10.18		20			17.10.19
14	1	8.1.19	155		19	1	23.1.19	170	
	2	8.1.19	155			2	23.1.19	170	
	3	8.1.19	155			3	23.1.19	170	
	4	8.1.19	155			4	30.1.19	177	
	5	15.1.19	162			5	30.1.19	177	
	6	15.1.19	162			6	30.1.19	177	
	7	22.1.19	169			7	30.1.19	177	
	8	29.1.19	176			8	30.1.19	177	
	9	29.1.19	176			9	30.1.19	177	
	10	29.1.19	176			10	6.2.19	184	
	11	29.1.19	176			11	6.2.19	184	
	12	29.1.19	176			12	6.2.19	184	
	13	29.1.19	176			13	6.2.19	184	
	14	29.1.19	176			14	13.2.19	191	
	15	29.1.19	176			15	13.2.19	191	
	16	5.2.19	183			16	20.2.19	198	
	17	5.2.19	183			17	20.2.19	198	
	18	10.3.19	216			18	20.2.19	198	
	19			30.10.18		19	6.3.19	212	
	20			25.9.18		20	6.3.19	212	
15	1	22.1.19	169		20	1	16.1.19	163	
	2	22.1.19	169			2	23.1.19	170	
	3	22.1.19	169			3	23.1.19	170	
	4	29.1.19	176			4	30.1.19	177	
	5	29.1.19	176			5	30.1.19	177	
	6	29.1.19	176			6	30.1.19	177	
	7	29.1.19	176			7	30.1.19	177	
	8	29.1.19	176			8	30.1.19	177	
	9	29.1.19	176			9	6.2.19	184	
	10	29.1.19	176			10	6.2.19	184	
	11	29.1.19	176			11	6.2.19	184	
	12	5.2.19	183			12	6.2.19	184	
	13	5.2.19	183			13	13.2.19	191	
	14	12.2.19	190			14	27.2.19	205	
	15	12.2.19	190			15	27.2.19	205	
	16	12.2.19	190			16	6.3.19	212	
	17	19.2.19	197			17			24.10.18
	18	19.2.19	197			18			10.10.18
	19	19.2.19	197			19			3.10.18
	20	19.2.19	197			20	Alive instar 11		

Table A5
Incubator 3 (I3), 20°C, 24D, mean hatch day 08.08.2018, developmental time (Dt) in days and pupation day or day of death (Dead larvae) of every larva.

Box (n)	Larva	Pupation day	Dt (days)	Dead larvae	Box (n)	Larva	Pupation day	Dt (days)	Dead larvae
21	1	22.1.19	167		26	1	5.2.19	181	
	2	29.1.19	174			2	5.2.19	181	
	3	5.2.19	181			3	5.2.19	181	
	4	5.2.19	181			4	5.2.19	181	
	5	5.2.19	181			5	12.2.19	188	
	6	5.2.19	181			6	12.2.19	188	
	7	5.2.19	181			7	19.2.19	195	
	8	12.2.19	188			8	19.2.19	195	
	9	12.2.19	188			9	19.2.19	195	
	10	12.2.19	188			10	19.2.19	195	
	11	12.2.19	188			11	19.2.19	195	
	12	12.2.19	188			12	26.2.19	202	
	13	12.2.19	188			13	5.3.19	209	
	14	19.2.19	195			14	5.3.19	209	
	15	19.2.19	195			15	20.3.19	224	
	16	19.2.19	195			16			29.1.19
	17	19.2.19	195			17			8.1.19
	18	19.2.19	195			18			27.11.18
	19			9.10.18		19			1.10.18
	20	Alive instar 16				20			1.10.18
22	1	29.1.19	174		27	1	22.1.19	167	
	2	5.2.19	181			2	22.1.19	167	
	3	5.2.19	181			3	29.1.19	174	
	4	12.2.19	188			4	29.1.19	174	
	5	12.2.19	188			5	5.2.19	181	
	6	12.2.19	188			6	12.2.19	188	
	7	12.2.19	188			7	12.2.19	188	
	8	12.2.19	188			8	12.2.19	188	
	9	19.2.19	195			9	12.2.19	188	
	10	19.2.19	195			10	12.2.19	188	
	11	19.2.19	195			11	19.2.19	195	
	12	19.2.19	195			12	19.2.19	195	
	13	19.2.19	195			13	19.2.19	195	
	14	26.2.19	202			14	19.2.19	195	
	15	26.2.19	202			15	19.2.19	195	
	16	5.3.19	209			16	19.2.19	195	
	17	2.4.19	237			17	26.2.19	202	
	18	2.4.19	237			18	26.2.19	202	
	19			27.11.18		19	20.3.19	224	
	20	Alive instar 16				20			6.11.18
23	1	22.1.19	167		28	1	23.1.19	168	
	2	22.1.19	167			2	30.1.19	175	
	3	29.1.19	174			3	30.1.19	175	
	4	29.1.19	174			4	6.2.19	182	
	5	29.1.19	174			5	6.2.19	182	
	6	5.2.19	181			6	13.2.19	189	
	7	5.2.19	181			7	13.2.19	189	
	8	5.2.19	181			8	13.2.19	189	
	9	12.2.19	188			9	13.2.19	189	
	10	12.2.19	188			10	13.2.19	189	
	11	12.2.19	188			11	20.2.19	196	
	12	19.2.19	195			12	20.2.19	196	
	13	26.2.19	202			13	20.2.19	196	

	14	5.3.19	209			14	27.2.19	203	
	15	5.3.19	209			15	27.2.19	203	
	16	10.3.19	214			16	10.3.19	214	
	17			1.1.19		17	10.4.19	245	
	18			4.12.18		18			24.10.18
	19			22.10.18		19			10.10.18
	20			9.10.18		20	Alive instar 16		
24	1	29.1.19	174		29	1	23.1.19	168	
	2	29.1.19	174			2	23.1.19	168	
	3	5.2.19	181			3	30.1.19	175	
	4	5.2.19	181			4	30.1.19	175	
	5	5.2.19	181			5	30.1.19	175	
	6	5.2.19	181			6	30.1.19	175	
	7	5.2.19	181			7	6.2.19	182	
	8	5.2.19	181			8	6.2.19	182	
	9	12.2.19	188			9	6.2.19	182	
	10	12.2.19	188			10	6.2.19	182	
	11	19.2.19	195			11	6.2.19	182	
	12	19.2.19	195			12	6.2.19	182	
	13	19.2.19	195			13	13.2.19	189	
	14	19.2.19	195			14	13.2.19	189	
	15	19.2.19	195			15	13.2.19	189	
	16	5.3.19	209			16	13.2.19	189	
	17	5.3.19	209			17	13.2.19	189	
	18	10.3.19	214			18	13.2.19	189	
	19			6.11.18		19	20.2.19	196	
	20			16.10.18		20			10.10.18
25	1	29.1.19	174		30	1	23.1.19	168	
	2	29.1.19	174			2	23.1.19	168	
	3	29.1.19	174			3	23.1.19	168	
	4	5.2.19	181			4	30.1.19	175	
	5	5.2.19	181			5	30.1.19	175	
	6	12.2.19	188			6	30.1.19	175	
	7	12.2.19	188			7	30.1.19	175	
	8	12.2.19	188			8	30.1.19	175	
	9	12.2.19	188			9	6.2.19	182	
	10	12.2.19	188			10	6.2.19	182	
	11	19.2.19	195			11	6.2.19	182	
	12	19.2.19	195			12	6.2.19	182	
	13	19.2.19	195			13	6.2.19	182	
	14	26.2.19	202			14	6.2.19	182	
	15	26.2.19	202			15	13.2.19	189	
	16	5.3.19	209			16	13.2.19	189	
	17	5.3.19	209			17	13.2.19	189	
	18	5.3.19	209			18	20.2.19	196	
	19			13.11.18		19	20.2.19	196	
	20			9.10.18		20	Larva lost		

Table A6
Incubator 4 (I4), 25°C, 16L/8D, mean hatch day 30.07.2018, developmental time (Dt) in days and pupation day or day of death (Dead larvae) of every larva.

Box (n)	Larva	Pupation day	Dt (days)	Dead larvae	Box (n)	Larva	Pupation day	Dt (days)	Dead larvae
31	1	17.11.18	110		36	1	25.11.18	118	
	2	1.12.18	124			2	2.12.18	125	

	3	8.12.18	131			3	2.12.18	125
	4	8.12.18	131			4	9.12.18	132
	5	8.12.18	131			5	9.12.18	132
	6	8.12.18	131			6	9.12.18	132
	7	8.12.18	131			7	9.12.18	132
	8	8.12.18	131			8	9.12.18	132
	9	15.12.18	138			9	16.12.18	139
	10	15.12.18	138			10	16.12.18	139
	11	22.12.18	145			11	23.12.18	146
	12	22.12.18	145			12	30.12.18	153
	13	22.12.18	145			13	5.1.19	159
	14	22.12.18	145			14	12.1.19	166
	15	22.12.18	145			15	12.1.19	166
	16	22.12.18	145			16	19.1.19	173
	17	29.12.18	152			17	19.1.19	173
	18	2.2.19	187			18	26.1.19	180
	19			29.12.18		19	10.3.19	223
	20			29.9.18		20	30.3.19	243
32	1	24.11.18	117		37	1	26.11.18	119
	2	24.11.18	117			2	26.11.18	119
	3	1.12.18	124			3	26.11.18	119
	4	1.12.18	124			4	26.11.18	119
	5	9.12.18	132			5	3.12.18	126
	6	9.12.18	132			6	3.12.18	126
	7	9.12.18	132			7	3.12.18	126
	8	9.12.18	132			8	3.12.18	126
	9	15.12.18	138			9	3.12.18	126
	10	15.12.18	138			10	3.12.18	126
	11	15.12.18	138			11	3.12.18	126
	12	22.12.18	145			12	9.12.18	132
	13	22.12.18	145			13	9.12.18	132
	14	22.12.18	145			14	9.12.18	132
	15	22.12.18	145			15	17.12.18	140
	16	29.12.18	152			16	17.12.18	140
	17	5.1.19	159			17	24.12.18	147
	18	19.1.19	173			18	31.12.18	154
	19			10.11.18		19	31.12.18	154
	20			10.11.18		20	7.1.19	161
33	1	1.12.18	124		38	1	3.12.18	126
	2	1.12.18	124			2	3.12.18	126
	3	1.12.18	124			3	3.12.18	126
	4	1.12.18	124			4	10.12.18	133
	5	1.12.18	124			5	10.12.18	133
	6	1.12.18	124			6	10.12.18	133
	7	9.12.18	132			7	17.12.18	140
	8	9.12.18	132			8	17.12.18	140
	9	9.12.18	132			9	17.12.18	140
	10	9.12.18	132			10	24.12.18	147
	11	15.12.18	138			11	24.12.18	147
	12	15.12.18	138			12	24.12.18	147
	13	15.12.18	138			13	24.12.18	147
	14	22.12.18	145			14	24.12.18	147
	15	22.12.18	145			15	31.12.18	154
	16	22.12.18	145			16	31.12.18	154
	17	22.12.18	145			17	31.12.18	154
	18	22.12.18	145			18	31.12.18	154
	19			13.10.18		19	7.1.19	161
	20			15.9.18		20	11.2.19	196
34	1	18.11.18	111		39	1	26.11.18	119

	2	25.11.18	118			2	24.12.18	147	
	3	25.11.18	118			3	24.12.18	147	
	4	2.12.18	125			4	24.12.18	147	
	5	2.12.18	125			5	24.12.18	147	
	6	9.12.18	132			6	24.12.18	147	
	7	9.12.18	132			7	24.12.18	147	
	8	9.12.18	132			8	24.12.18	147	
	9	9.12.18	132			9	24.12.18	147	
	10	9.12.18	132			10	24.12.18	147	
	11	16.12.18	139			11	24.12.18	147	
	12	23.12.18	146			12	24.12.18	147	
	13	30.12.18	153			13	24.12.18	147	
	14	30.12.18	153			14	24.12.18	147	
	15	30.12.18	153			15	31.12.18	154	
	16	30.12.18	153			16	7.1.19	161	
	17	12.1.19	166			17	21.1.19	175	
	18	19.1.19	173			18	21.1.19	175	
	19	9.2.19	194			19	21.1.19	175	
	20	9.2.19	194			20	4.2.19	189	
35	1	2.12.18	125		40	1	3.12.18	126	
	2	2.12.18	125			2	3.12.18	126	
	3	2.12.18	125			3	3.12.18	126	
	4	2.12.18	125			4	3.12.18	126	
	5	2.12.18	125			5	10.12.18	133	
	6	9.12.18	132			6	10.12.18	133	
	7	9.12.18	132			7	10.12.18	133	
	8	9.12.18	132			8	10.12.18	133	
	9	9.12.18	132			9	10.12.18	133	
	10	9.12.18	132			10	17.12.18	140	
	11	9.12.18	132			11	17.12.18	140	
	12	16.12.18	139			12	17.12.18	140	
	13	16.12.18	139			13	17.12.18	140	
	14	23.12.18	146			14	17.12.18	140	
	15	23.12.18	146			15	17.12.18	140	
	16	30.12.18	153			16	24.12.18	147	
	17	30.12.18	153			17	24.12.18	147	
	18	30.12.18	153			18	24.12.18	147	
	19	12.1.19	166			19	24.12.18	147	
	20			2.9.18		20	24.12.18	147	

Table A7
Incubator 5 (I5), 25°C, 8L/16D, mean hatch day 29.07.2018, developmental time (Dt) in days and pupation day or day of death (Dead larvae) of every larva.

Box (n)	Larva	Pupation day	Dt (days)	Dead larvae	Box (n)	Larva	Pupation day	Dt (days)	Dead larvae
41	1	1.12.18	125		46	1	2.12.18	126	
	2	1.12.18	125			2	9.12.18	133	
	3	9.12.18	133			3	9.12.18	133	
	4	9.12.18	133			4	9.12.18	133	
	5	9.12.18	133			5	16.12.18	140	
	6	9.12.18	133			6	16.12.18	140	
	7	9.12.18	133			7	16.12.18	140	
	8	15.12.18	139			8	16.12.18	140	
	9	15.12.18	139			9	16.12.18	140	
	10	15.12.18	139			10	16.12.18	140	

	11	22.12.18	146			11	23.12.18	147	
	12	22.12.18	146			12	23.12.18	147	
	13	22.12.18	146			13	23.12.18	147	
	14	22.12.18	146			14	23.12.18	147	
	15	22.12.18	146			15	30.12.18	154	
	16	29.12.18	153			16	30.12.18	154	
	17	29.12.18	153			17	5.1.19	160	
	18	29.12.18	153			18	5.1.19	160	
	19	5.1.19	160			19	5.1.19	160	
	20	12.1.19	167			20	19.1.19	174	
42	1	1.12.18	125		47	1	3.12.18	127	
	2	9.12.18	133			2	3.12.18	127	
	3	9.12.18	133			3	3.12.18	127	
	4	9.12.18	133			4	3.12.18	127	
	5	9.12.18	133			5	3.12.18	127	
	6	15.12.18	139			6	10.12.18	134	
	7	15.12.18	139			7	10.12.18	134	
	8	15.12.18	139			8	10.12.18	134	
	9	15.12.18	139			9	10.12.18	134	
	10	15.12.18	139			10	17.12.18	141	
	11	22.12.18	146			11	24.12.18	148	
	12	22.12.18	146			12	24.12.18	148	
	13	22.12.18	146			13	24.12.18	148	
	14	22.12.18	146			14	31.12.18	155	
	15	22.12.18	146			15	31.12.18	155	
	16	22.12.18	146			16	31.12.18	155	
	17	22.12.18	146			17	7.1.19	162	
	18	29.12.18	153			18	21.1.19	176	
	19			27.10.18		19	4.2.19	190	
	20			1.9.18		20	4.3.19	218	
43	1	9.12.18	133		48	1	3.12.18	127	
	2	9.12.18	133			2	3.12.18	127	
	3	15.12.18	139			3	10.12.18	134	
	4	15.12.18	139			4	10.12.18	134	
	5	22.12.18	146			5	17.12.18	141	
	6	22.12.18	146			6	17.12.18	141	
	7	22.12.18	146			7	17.12.18	141	
	8	29.12.18	153			8	24.12.18	148	
	9	29.12.18	153			9	24.12.18	148	
	10	29.12.18	153			10	24.12.18	148	
	11	29.12.18	153			11	24.12.18	148	
	12	5.1.19	160			12	24.12.18	148	
	13	5.1.19	160			13	31.12.18	155	
	14	5.1.19	160			14	31.12.18	155	
	15	5.1.19	160			15	14.1.19	169	
	16	5.1.19	160			16	28.1.19	183	
	17	5.1.19	160			17	11.2.19	197	
	18	19.1.19	174			18			19.11.18
	19	19.1.19	174			19			29.10.18
	20			15.9.18		20			3.9.18
44	1	2.12.18	126		49	1	3.12.18	127	
	2	2.12.18	126			2	3.12.18	127	
	3	2.12.18	126			3	10.12.18	134	
	4	2.12.18	126			4	10.12.18	134	
	5	9.12.18	133			5	17.12.18	141	
	6	9.12.18	133			6	17.12.18	141	
	7	9.12.18	133			7	17.12.18	141	
	8	9.12.18	133			8	24.12.18	148	
	9	9.12.18	133			9	24.12.18	148	

	10	9.12.18	133			10	24.12.18	148	
	11	16.12.18	140			11	24.12.18	148	
	12	16.12.18	140			12	24.12.18	148	
	13	16.12.18	140			13	31.12.18	155	
	14	16.12.18	140			14	31.12.18	155	
	15	16.12.18	140			15	31.12.18	155	
	16	16.12.18	140			16	31.12.18	155	
	17	23.12.18	147			17	7.1.19	162	
	18	23.12.18	147			18	14.1.19	169	
	19	23.12.18	147			19	21.1.19	176	
	20	5.1.19	160			20	28.1.19	183	
45	1	2.12.18	126		50	1	3.12.18	127	
	2	9.12.18	133			2	10.12.18	134	
	3	9.12.18	133			3	10.12.18	134	
	4	16.12.18	140			4	10.12.18	134	
	5	16.12.18	140			5	10.12.18	134	
	6	23.12.18	147			6	10.12.18	134	
	7	23.12.18	147			7	10.12.18	134	
	8	23.12.18	147			8	10.12.18	134	
	9	23.12.18	147			9	10.12.18	134	
	10	23.12.18	147			10	17.12.18	141	
	11	30.12.18	154			11	17.12.18	141	
	12	30.12.18	154			12	17.12.18	141	
	13	30.12.18	154			13	24.12.18	148	
	14	30.12.18	154			14	24.12.18	148	
	15	5.1.19	160			15	24.12.18	148	
	16	12.1.19	167			16	7.1.19	162	
	17	19.1.19	174			17	14.1.19	169	
	18	16.2.19	202			18	21.1.19	176	
	19	10.3.19	224			19	25.2.19	211	
	20			2.9.18		20			8.10.18

Table A8
Incubator 6 (I6), 25°C, 24D, mean hatch day 29.07.2018, developmental time (Dt) in days and pupation day or day of death (Dead larvae) of every larva.

Box (n)	Larva	Pupation day	Dt (days)	Dead larvae	Box (n)	Larva	Pupation day	Dt (days)	Dead larvae
51	1	17.11.18	111		56	1	11.11.18	105	
	2	17.11.18	111			2	18.11.18	112	
	3	17.11.18	111			3	18.11.18	112	
	4	17.11.18	111			4	18.11.18	112	
	5	24.11.18	118			5	18.11.18	112	
	6	24.11.18	118			6	18.11.18	112	
	7	24.11.18	118			7	25.11.18	119	
	8	1.12.18	125			8	25.11.18	119	
	9	1.12.18	125			9	25.11.18	119	
	10	1.12.18	125			10	25.11.18	119	
	11	1.12.18	125			11	25.11.18	119	
	12	1.12.18	125			12	25.11.18	119	
	13	9.12.18	133			13	25.11.18	119	
	14	9.12.18	133			14	25.11.18	119	
	15	9.12.18	133			15	2.12.18	126	
	16	9.12.18	133			16	2.12.18	126	
	17	9.12.18	133			17	2.12.18	126	
	18	9.12.18	133			18	2.12.18	126	

	19	22.12.18	146			19	9.12.18	133
	20	5.1.19	160			20	16.12.18	140
52	1	17.11.18	111		57	1	19.11.18	113
	2	17.11.18	111			2	19.11.18	113
	3	17.11.18	111			3	19.11.18	113
	4	17.11.18	111			4	26.11.18	120
	5	24.11.18	118			5	26.11.18	120
	6	24.11.18	118			6	26.11.18	120
	7	24.11.18	118			7	3.12.18	127
	8	24.11.18	118			8	3.12.18	127
	9	1.12.18	125			9	3.12.18	127
	10	1.12.18	125			10	3.12.18	127
	11	1.12.18	125			11	3.12.18	127
	12	1.12.18	125			12	3.12.18	127
	13	1.12.18	125			13	3.12.18	127
	14	1.12.18	125			14	10.12.18	134
	15	1.12.18	125			15	10.12.18	134
	16	1.12.18	125			16	10.12.18	134
	17	1.12.18	125			17	10.12.18	134
	18	9.12.18	133			18	10.12.18	134
	19	22.12.18	146			19	10.12.18	134
	20			29.9.18		20	Alive instar 12	
53	1	17.11.18	111		58	1	19.11.18	113
	2	24.11.18	118			2	19.11.18	113
	3	24.11.18	118			3	19.11.18	113
	4	24.11.18	118			4	26.11.18	120
	5	1.12.18	125			5	26.11.18	120
	6	1.12.18	125			6	26.11.18	120
	7	1.12.18	125			7	26.11.18	120
	8	1.12.18	125			8	26.11.18	120
	9	1.12.18	125			9	26.11.18	120
	10	1.12.18	125			10	26.11.18	120
	11	1.12.18	125			11	3.12.18	127
	12	1.12.18	125			12	3.12.18	127
	13	1.12.18	125			13	3.12.18	127
	14	1.12.18	125			14	3.12.18	127
	15	9.12.18	133			15	3.12.18	127
	16	9.12.18	133			16	3.12.18	127
	17	9.12.18	133			17	10.12.18	134
	18	9.12.18	133			18	10.12.18	134
	19	15.12.18	139			19	10.12.18	134
	20			19.1.19		20	10.12.18	134
54	1	11.11.18	105		59	1	3.12.18	127
	2	18.11.18	112			2	3.12.18	127
	3	25.11.18	119			3	3.12.18	127
	4	25.11.18	119			4	3.12.18	127
	5	25.11.18	119			5	11.12.18	135
	6	25.11.18	119			6	11.12.18	135
	7	25.11.18	119			7	11.12.18	135
	8	25.11.18	119			8	11.12.18	135
	9	25.11.18	119			9	11.12.18	135
	10	2.12.18	126			10	11.12.18	135
	11	2.12.18	126			11	11.12.18	135
	12	2.12.18	126			12	11.12.18	135
	13	2.12.18	126			13	11.12.18	135
	14	2.12.18	126			14	11.12.18	135
	15	9.12.18	133			15	17.12.18	141
	16	9.12.18	133			16	17.12.18	141
	17	16.12.18	140			17	17.12.18	141

	18	23.12.18	147			18	17.12.18	141	
	19	23.12.18	147			19	17.12.18	141	
	20	30.12.18	154			20	24.12.18	148	
55	1	11.11.18	105		60	1	19.11.18	113	
	2	18.11.18	112			2	19.11.18	113	
	3	25.11.18	119			3	19.11.18	113	
	4	25.11.18	119			4	26.11.18	120	
	5	25.11.18	119			5	3.12.18	127	
	6	25.11.18	119			6	3.12.18	127	
	7	25.11.18	119			7	3.12.18	127	
	8	25.11.18	119			8	3.12.18	127	
	9	25.11.18	119			9	3.12.18	127	
	10	25.11.18	119			10	3.12.18	127	
	11	25.11.18	119			11	3.12.18	127	
	12	2.12.18	126			12	3.12.18	127	
	13	2.12.18	126			13	3.12.18	127	
	14	2.12.18	126			14	11.12.18	135	
	15	2.12.18	126			15	11.12.18	135	
	16	9.12.18	133			16	11.12.18	135	
	17	9.12.18	133			17	11.12.18	135	
	18	9.12.18	133			18	11.12.18	135	
	19	9.12.18	133			19	17.12.18	141	
	20	9.12.18	133			20			3.9.18

Table A9
Incubator 7 (I7), 30°C, 16L/8D, mean hatch day 26.07.2018, developmental time (Dt) in days and pupation day or day of death (Dead larvae) of every larva.

Box (n)	Larva	Pupation day	Dt (days)	Dead larvae	Box (n)	Larva	Pupation day	Dt (days)	Dead larvae
61	1	14.11.18	111		66	1	29.11.18	126	
	2	21.11.18	118			2	29.11.18	126	
	3	21.11.18	118			3	29.11.18	126	
	4	21.11.18	118			4	29.11.18	126	
	5	21.11.18	118			5	29.11.18	126	
	6	28.11.18	125			6	29.11.18	126	
	7	28.11.18	125			7	29.11.18	126	
	8	28.11.18	125			8	6.12.18	133	
	9	28.11.18	125			9	6.12.18	133	
	10	28.11.18	125			10	6.12.18	133	
	11	28.11.18	125			11	6.12.18	133	
	12	28.11.18	125			12	6.12.18	133	
	13	28.11.18	125			13	13.12.18	140	
	14	5.12.18	132			14	20.12.18	147	
	15	5.12.18	132			15	20.12.18	147	
	16	5.12.18	132			16	20.12.18	147	
	17	26.12.18	153			17	20.12.18	147	
	18	23.1.19	181			18	26.12.18	153	
	19	20.3.19	237			19	9.1.19	167	
	20			9.1.19		20	6.2.19	195	
62	1	14.11.18	111		67	1	15.11.18	112	
	2	21.11.18	118			2	29.11.18	126	
	3	21.11.18	118			3	29.11.18	126	
	4	21.11.18	118			4	29.11.18	126	
	5	28.11.18	125			5	29.11.18	126	
	6	5.12.18	132			6	6.12.18	133	

	7	5.12.18	132			7	13.12.18	140	
	8	12.12.18	139			8	20.12.18	147	
	9	12.12.18	139			9	20.12.18	147	
	10	12.12.18	139			10	2.1.19	160	
	11	19.12.18	146			11	2.1.19	160	
	12	26.12.18	153			12	16.1.19	174	
	13	2.1.19	160			13	20.2.19	209	
	14	9.1.19	167			14			23.1.19
	15	9.1.19	167			15			20.9.18
	16	9.1.19	167			16			14.9.18
	17	23.1.19	181			17			6.9.18
	18	30.1.19	188			18	Larva lost		
	19	30.1.19	188			19	Larva lost		
	20			6.9.18		20	Larva lost		
63	1	14.11.18	111		68	1	8.11.18	105	
	2	21.11.18	118			2	15.11.18	112	
	3	21.11.18	118			3	22.11.18	119	
	4	28.11.18	125			4	22.11.18	119	
	5	28.11.18	125			5	22.11.18	119	
	6	28.11.18	125			6	22.11.18	119	
	7	28.11.18	125			7	22.11.18	119	
	8	5.12.18	132			8	22.11.18	119	
	9	5.12.18	132			9	22.11.18	119	
	10	5.12.18	132			10	29.11.18	126	
	11	5.12.18	132			11	29.11.18	126	
	12	12.12.18	139			12	29.11.18	126	
	13	12.12.18	139			13	29.11.18	126	
	14	12.12.18	139			14	29.11.18	126	
	15	19.12.18	146			15	6.12.18	133	
	16	19.12.18	146			16	13.12.18	140	
	17	19.12.18	146			17	13.12.18	140	
	18	2.1.19	160			18	26.12.18	153	
	19	16.1.19	174			19	26.12.18	153	
	20			30.1.19		20	26.12.18	153	
64	1	21.11.18	118		69	1	22.11.18	119	
	2	21.11.18	118			2	22.11.18	119	
	3	28.11.18	125			3	22.11.18	119	
	4	28.11.18	125			4	22.11.18	119	
	5	5.12.18	132			5	29.11.18	126	
	6	5.12.18	132			6	29.11.18	126	
	7	5.12.18	132			7	29.11.18	126	
	8	5.12.18	132			8	6.12.18	133	
	9	5.12.18	132			9	6.12.18	133	
	10	5.12.18	132			10	6.12.18	133	
	11	5.12.18	132			11	6.12.18	133	
	12	13.12.18	140			12	13.12.18	140	
	13	13.12.18	140			13	13.12.18	140	
	14	19.12.18	146			14	20.12.18	147	
	15	19.12.18	146			15	20.12.18	147	
	16	19.12.18	146			16	20.12.18	147	
	17	19.12.18	146			17	20.12.18	147	
	18	26.12.18	153			18	20.12.18	147	
	19	26.12.18	153			19	26.12.18	153	
	20	Alive instar 16				20			31.8.18
65	1	14.11.18	111		70	1	22.11.18	119	
	2	14.11.18	111			2	29.11.18	126	
	3	21.11.18	118			3	6.12.18	133	
	4	21.11.18	118			4	13.12.18	140	
	5	28.11.18	125			5	13.12.18	140	

	6	28.11.18	125			6	13.12.18	140	
	7	28.11.18	125			7	13.12.18	140	
	8	5.12.18	132			8	20.12.18	147	
	9	5.12.18	132			9	20.12.18	147	
	10	13.12.18	140			10	26.12.18	153	
	11	13.12.18	140			11	2.1.19	160	
	12	13.12.18	140			12	9.1.19	167	
	13	13.12.18	140			13	9.1.19	167	
	14	19.12.18	146			14	16.1.19	174	
	15	26.12.18	153			15	23.1.19	181	
	16	2.1.19	160			16	6.2.19	195	
	17	9.1.19	167			17	20.3.19	237	
	18	16.1.19	174			18			22.11.18
	19	23.1.19	181			19			14.9.18
	20	6.3.19	223			20			31.8.18

Table A10
Incubator 8 (I8), 30°C, 8L/16D, mean hatch day 29.07.2018, developmental time (Dt) in days and pupation day or day of death (Dead larvae) of every larva.

Box (n)	Larva	Pupation day	Dt (days)	Dead larvae	Box (n)	Larva	Pupation day	Dt (days)	Dead larvae
71	1	14.11.18	108		76	1	22.11.18	116	
	2	21.11.18	115			2	29.11.18	123	
	3	21.11.18	115			3	29.11.18	123	
	4	21.11.18	115			4	29.11.18	123	
	5	21.11.18	115			5	6.12.18	130	
	6	28.11.18	122			6	13.12.18	137	
	7	28.11.18	122			7	20.12.18	144	
	8	28.11.18	122			8	26.12.18	150	
	9	28.11.18	122			9	2.1.19	157	
	10	28.11.18	122			10	9.1.19	164	
	11	28.11.18	122			11	9.1.19	164	
	12	28.11.18	122			12	16.1.19	171	
	13	5.12.18	129			13	16.1.19	171	
	14	5.12.18	129			14	23.1.19	178	
	15	5.12.18	129			15	23.1.19	178	
	16	12.12.18	136			16	30.1.19	185	
	17	12.12.18	136			17	30.1.19	185	
	18	12.12.18	136			18	30.1.19	185	
	19	26.12.18	150			19	6.2.19	192	
	20	26.12.18	150			20	Alive instar 16		
72	1	21.11.18	115		77	1	22.11.18	116	
	2	28.11.18	122			2	22.11.18	116	
	3	28.11.18	122			3	22.11.18	116	
	4	5.12.18	129			4	22.11.18	116	
	5	5.12.18	129			5	22.11.18	116	
	6	12.12.18	136			6	29.11.18	123	
	7	12.12.18	136			7	29.11.18	123	
	8	12.12.18	136			8	29.11.18	123	
	9	12.12.18	136			9	6.12.18	130	
	10	19.12.18	143			10	6.12.18	130	
	11	19.12.18	143			11	6.12.18	130	
	12	26.12.18	150			12	6.12.18	130	
	13	26.12.18	150			13	6.12.18	130	
	14	26.12.18	150			14	6.12.18	130	

	15	2.1.19	157			15	13.12.18	137	
	16	9.1.19	164			16	13.12.18	137	
	17	23.1.19	178			17	13.12.18	137	
	18	30.1.19	185			18	20.12.18	144	
	19			24.10.18		19	2.1.19	157	
	20	Larva lost				20	9.1.19	164	
73	1	21.11.18	115		78	1	15.11.18	109	
	2	21.11.18	115			2	15.11.18	109	
	3	21.11.18	115			3	22.11.18	116	
	4	28.11.18	122			4	22.11.18	116	
	5	28.11.18	122			5	22.11.18	116	
	6	28.11.18	122			6	29.11.18	123	
	7	28.11.18	122			7	29.11.18	123	
	8	28.11.18	122			8	29.11.18	123	
	9	28.11.18	122			9	29.11.18	123	
	10	28.11.18	122			10	29.11.18	123	
	11	28.11.18	122			11	29.11.18	123	
	12	28.11.18	122			12	6.12.18	130	
	13	5.12.18	129			13	6.12.18	130	
	14	5.12.18	129			14	6.12.18	130	
	15	5.12.18	129			15	6.12.18	130	
	16	5.12.18	129			16	13.12.18	137	
	17	5.12.18	129			17	13.12.18	137	
	18	12.12.18	136			18	13.12.18	137	
	19	26.12.18	150			19	13.12.18	137	
	20	2.1.19	157			20			20.2.19
74	1	21.11.18	115		79	1	15.11.18	109	
	2	28.11.18	122			2	15.11.18	109	
	3	28.11.18	122			3	15.11.18	109	
	4	28.11.18	122			4	22.11.18	116	
	5	28.11.18	122			5	29.11.18	123	
	6	28.11.18	122			6	29.11.18	123	
	7	28.11.18	122			7	29.11.18	123	
	8	28.11.18	122			8	29.11.18	123	
	9	28.11.18	122			9	29.11.18	123	
	10	13.12.18	137			10	29.11.18	123	
	11	13.12.18	137			11	29.11.18	123	
	12	13.12.18	137			12	29.11.18	123	
	13	13.12.18	137			13	29.11.18	123	
	14	13.12.18	137			14	29.11.18	123	
	15	19.12.18	143			15	6.12.18	130	
	16	26.12.18	150			16	6.12.18	130	
	17	26.12.18	150			17	13.12.18	137	
	18	26.12.18	150			18	13.12.18	137	
	19	16.1.19	171			19	20.12.18	144	
	20			31.10.18		20	2.1.19	157	
75	1	14.11.18	108		80	1	22.11.18	116	
	2	21.11.18	115			2	22.11.18	116	
	3	21.11.18	115			3	22.11.18	116	
	4	28.11.18	122			4	22.11.18	116	
	5	28.11.18	122			5	22.11.18	116	
	6	28.11.18	122			6	29.11.18	123	
	7	28.11.18	122			7	29.11.18	123	
	8	28.11.18	122			8	29.11.18	123	
	9	5.12.18	129			9	6.12.18	130	
	10	5.12.18	129			10	6.12.18	130	
	11	5.12.18	129			11	6.12.18	130	
	12	5.12.18	129			12	6.12.18	130	
	13	5.12.18	129			13	6.12.18	130	

	14	13.12.18	137			14	6.12.18	130
	15	13.12.18	137			15	6.12.18	130
	16	13.12.18	137			16	13.12.18	137
	17	13.12.18	137			17	20.12.18	144
	18	26.12.18	150			18	20.12.18	144
	19			26.9.18		19	20.12.18	144
	20			13.9.18		20	30.1.19	185

Table A11
Incubator 9 (I9), 30°C, 24D, mean hatch day 30.07.2018, developmental time (Dt) in days and pupation day or day of death (Dead larvae) of every larva.

Box (n)	Larva	Pupation day	Dt (days)	Dead larvae	Box (n)	Larva	Pupation day	Dt (days)	Dead larvae
81	1	24.11.18	117		86	1	18.11.18	111	
	2	24.11.18	117			2	25.11.18	118	
	3	24.11.18	117			3	25.11.18	118	
	4	1.12.18	124			4	25.11.18	118	
	5	1.12.18	124			5	2.12.18	125	
	6	1.12.18	124			6	2.12.18	125	
	7	1.12.18	124			7	2.12.18	125	
	8	8.12.18	131			8	9.12.18	132	
	9	8.12.18	131			9	9.12.18	132	
	10	15.12.18	138			10	9.12.18	132	
	11	15.12.18	138			11	9.12.18	132	
	12	15.12.18	138			12	9.12.18	132	
	13	5.1.19	159			13	9.12.18	132	
	14	19.1.19	173			14	9.12.18	132	
	15	19.1.19	173			15	23.12.18	146	
	16	2.2.19	187			16	23.12.18	146	
	17	23.2.19	208			17	23.12.18	146	
	18	27.4.19	271			18	30.12.18	153	
	19			14.5.19		19	30.12.18	153	
	20			27.4.19		20	30.12.18	153	
82	1	17.11.18	110		87	1	19.11.18	112	
	2	24.11.18	117			2	19.11.18	112	
	3	1.12.18	124			3	19.11.18	112	
	4	1.12.18	124			4	26.11.18	119	
	5	1.12.18	124			5	26.11.18	119	
	6	9.12.18	132			6	3.12.18	126	
	7	9.12.18	132			7	10.12.18	133	
	8	9.12.18	132			8	10.12.18	133	
	9	9.12.18	132			9	17.12.18	140	
	10	9.12.18	132			10	17.12.18	140	
	11	9.12.18	132			11	17.12.18	140	
	12	9.12.18	132			12	24.12.18	147	
	13	9.12.18	132			13	24.12.18	147	
	14	9.12.18	132			14	24.12.18	147	
	15	15.12.18	138			15	31.12.18	154	
	16	22.12.18	145			16	31.12.18	154	
	17	22.12.18	145			17	31.12.18	154	
	18	29.12.18	152			18	7.1.19	161	
	19	2.2.19	187			19	14.1.19	168	
	20	2.3.19	215			20	4.2.19	189	
83	1	24.11.18	117		88	1	19.11.18	112	
	2	1.12.18	124			2	19.11.18	112	

	3	8.12.18	131		3	26.11.18	119	
	4	8.12.18	131		4	3.12.18	126	
	5	8.12.18	131		5	3.12.18	126	
	6	8.12.18	131		6	3.12.18	126	
	7	8.12.18	131		7	10.12.18	133	
	8	15.12.18	138		8	10.12.18	133	
	9	15.12.18	138		9	10.12.18	133	
	10	15.12.18	138		10	10.12.18	133	
	11	15.12.18	138		11	10.12.18	133	
	12	15.12.18	138		12	10.12.18	133	
	13	15.12.18	138		13	17.12.18	140	
	14	15.12.18	138		14	17.12.18	140	
	15	15.12.18	138		15	17.12.18	140	
	16	29.12.18	152		16	17.12.18	140	
	17	29.12.18	152		17	24.12.18	147	
	18	5.1.19	159		18	24.12.18	147	
	19	19.1.19	173		19	24.12.18	147	
	20	26.1.19	180		20	4.2.19	189	
84	1	18.11.18	111	89	1	19.11.18	112	
	2	25.11.18	118		2	19.11.18	112	
	3	25.11.18	118		3	26.11.18	119	
	4	25.11.18	118		4	26.11.18	119	
	5	25.11.18	118		5	26.11.18	119	
	6	25.11.18	118		6	3.12.18	126	
	7	25.11.18	118		7	3.12.18	126	
	8	25.11.18	118		8	3.12.18	126	
	9	25.11.18	118		9	3.12.18	126	
	10	2.12.18	125		10	3.12.18	126	
	11	2.12.18	125		11	10.12.18	133	
	12	2.12.18	125		12	10.12.18	133	
	13	2.12.18	125		13	10.12.18	133	
	14	9.12.18	132		14	17.12.18	140	
	15	9.12.18	132		15	17.12.18	140	
	16	9.12.18	132		16	17.12.18	140	
	17	9.12.18	132		17	24.12.18	147	
	18	23.12.18	146		18	24.12.18	147	
	19	5.1.19	159		19	21.1.19	175	
	20	12.1.19	166		20	10.3.19	223	
85	1	11.11.18	104	90	1	26.11.18	119	
	2	11.11.18	104		2	26.11.18	119	
	3	25.11.18	118		3	26.11.18	119	
	4	25.11.18	118		4	3.12.18	126	
	5	2.12.18	125		5	3.12.18	126	
	6	2.12.18	125		6	3.12.18	126	
	7	2.12.18	125		7	3.12.18	126	
	8	2.12.18	125		8	3.12.18	126	
	9	2.12.18	125		9	3.12.18	126	
	10	9.12.18	132		10	3.12.18	126	
	11	9.12.18	132		11	3.12.18	126	
	12	9.12.18	132		12	3.12.18	126	
	13	9.12.18	132		13	10.12.18	133	
	14	9.12.18	132		14	10.12.18	133	
	15	16.12.18	139		15	10.12.18	133	
	16	30.12.18	153		16	10.12.18	133	
	17	30.12.18	153		17	17.12.18	140	
	18	5.1.19	159		18	24.12.18	147	
	19	19.1.19	173		19	24.12.18	147	
	20	Alive instar 16			20			12.11.18

Table A12

Incubator 1 (I1), 20 °C, 16L/8D

Mean larval weight (MLW) in mg, growth rate (Gr) in % and weighted mean growth rate (WMGr) in % of every box during every week of data collection.

Week	Term	n									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
0	MLW (mg)	1.5	1.6	1.4	1.5	1.5	1.4	1.8	1.2	1.6	1.7
1	MLW (mg)	2.4	2.4	2.3	2.4	2.3	2.1	2.5	1.8	2.5	2.8
	Gr (%)	58.2%	48.1%	62.2%	64.2%	57.8%	53.5%	43.9%	50.4%	51.1%	64.3%
2	MLW (mg)	3.3	3.1	2.6	3.2	3.0	2.9	3.4	2.5	3.0	3.5
	Gr (%)	35.7%	30.4%	12.8%	33.0%	30.4%	39.0%	36.3%	33.9%	23.0%	26.2%
3	MLW (mg)	4.0	4.0	3.8	3.9	3.8	3.3	4.2	2.9	3.8	4.7
	Gr (%)	21.0%	28.7%	44.5%	23.0%	28.3%	13.7%	22.7%	18.5%	25.1%	31.8%
4	MLW (mg)	5.4	5.2	4.3	4.7	5.0	4.7	5.7	4.0	4.7	6.0
	Gr (%)	35.8%	28.9%	14.5%	20.0%	30.7%	42.2%	35.7%	36.2%	24.5%	29.3%
5	MLW (mg)	6.2	6.5	5.8	6.4	6.5	5.4	7.0	4.9	6.0	7.6
	Gr (%)	15.2%	25.6%	34.6%	36.7%	29.0%	14.5%	23.1%	22.9%	27.1%	25.7%
6	MLW (mg)	8.1	8.2	7.0	7.2	8.4	7.3	9.1	6.6	7.4	10.0
	Gr (%)	31.3%	25.8%	21.0%	12.9%	29.6%	35.5%	29.9%	35.7%	22.4%	31.8%
7	MLW (mg)	10.0	9.9	9.5	10.0	10.2	8.5	11.3	7.9	9.3	11.9
	Gr (%)	23.5%	21.1%	36.0%	37.9%	21.4%	17.5%	23.5%	19.9%	26.5%	19.6%
8	MLW (mg)	13.1	13.1	11.6	11.6	13.8	11.1	14.3	10.3	11.5	15.3
	Gr (%)	30.9%	32.3%	22.3%	16.4%	35.5%	30.8%	26.7%	29.9%	22.7%	28.5%
9	MLW (mg)	16.1	14.8	14.5	14.8	15.7	13.0	17.8	12.7	13.8	18.6
	Gr (%)	22.7%	13.1%	24.2%	27.2%	13.8%	16.2%	24.3%	22.8%	20.7%	21.6%
10	MLW (mg)	19.7	21.7	19.3	19.4	22.2	17.6	22.3	15.9	18.7	23.9
	Gr (%)	22.1%	46.2%	33.2%	31.0%	41.4%	35.5%	25.6%	25.4%	35.2%	27.9%
11	MLW (mg)	26.1	24.2	23.2	24.2	26.2	20.6	29.4	20.6	22.0	30.2
	Gr (%)	32.8%	11.9%	20.6%	25.2%	17.9%	17.3%	31.6%	29.9%	17.6%	26.8%
12	MLW (mg)	30.8	33.7	31.5	34.0	33.9	28.4	38.4	25.5	29.9	37.5
	Gr (%)	18.0%	39.1%	35.4%	40.4%	29.5%	38.0%	30.8%	23.4%	35.7%	23.8%
13	MLW (mg)	39.4	41.7	36.5	38.3	41.8	33.0	43.9	31.3	35.0	46.7
	Gr (%)	27.7%	23.6%	15.9%	12.5%	23.3%	16.1%	14.3%	22.9%	17.0%	24.7%
14	MLW (mg)	48.6	51.0	48.2	50.0	49.5	40.7	56.7	39.5	42.9	59.1
	Gr (%)	23.5%	22.4%	32.2%	30.7%	18.4%	23.2%	29.0%	26.3%	22.8%	26.6%
15	MLW (mg)	59.7	70.7	59.5	62.9	63.3	53.0	66.2	46.6	56.6	69.3
	Gr (%)	22.9%	38.5%	23.3%	25.9%	27.9%	30.2%	16.8%	17.9%	32.0%	17.2%
16	MLW (mg)	72.5	76.9	68.5	68.9	72.9	61.0	79.7	57.5	64.0	82.8
	Gr (%)	21.5%	8.8%	15.1%	9.5%	15.2%	15.2%	20.4%	23.3%	13.0%	19.5%
17	MLW (mg)	85.7	88.9	85.2	85.9	83.0	78.8	99.3	69.2	76.5	95.4
	Gr (%)	18.2%	15.6%	24.4%	24.7%	13.9%	29.1%	24.5%	20.4%	19.6%	15.2%
18	MLW (mg)	98.6	107.4	95.0	94.2	91.0	88.3	98.2	76.7	86.6	101.0
	Gr (%)	15.1%	20.8%	11.6%	9.7%	9.7%	12.2%	-1.1%	10.8%	13.2%	5.9%
19	MLW (mg)	110.2	113.7	101.4	94.1	92.3	98.0	99.3	82.9	92.7	112.1
	Gr (%)	11.7%	5.9%	6.7%	-0.1%	1.4%	10.9%	1.2%	8.1%	7.1%	11.0%
20	MLW (mg)	117.9	115.8	99.5	94.6	94.6	105.7	103.3	88.6	100.1	114.2
	Gr (%)	7.0%	1.8%	-1.9%	0.5%	2.5%	7.8%	4.0%	6.8%	7.9%	1.8%
21	MLW (mg)	114.4	140.4	88.1	100.4	91.7	98.6	96.3	87.1	104.1	112.8
	Gr (%)	-3.0%	21.3%	-11.5%	6.1%	-3.1%	-6.7%	-6.7%	-1.7%	4.0%	-1.2%
	WMGr (%)			**25.3%**							
22	MLW (mg)	111.6	132.5		103.2	92.1	99.7	79.9	85.3	103.0	98.4
	Gr (%)	-2.4%	-5.7%		2.8%	0.5%	1.1%	-17.1%	-2.1%	-1.0%	-12.7%
	WMGr (%)	**24.3%**	**25.5%**								
23	MLW (mg)				115.1	96.3	103.7	66.1	77.4	85.5	109.1
	Gr (%)				11.6%	4.5%	4.0%	-17.3%	-9.2%	-16.9%	10.8%
	WMGr (%)							**23.3%**			
24	MLW (mg)				125.8	105.2	88.4		91.3	72.0	101.0
	Gr (%)				9.2%	9.3%	-14.8%		17.9%	-15.8%	-7.4%
	WMGr (%)						**23.8%**			**22.1%**	

25	MLW (mg)		143.7	103.1	94.2	100.5
	Gr (%)		14.2%	-2.1%	3.1%	-0.5%
	WMGr (%)			**23.4%**		
26	MLW (mg)		168.5		79.0	101.1
	Gr (%)		17.3%		-16.1%	0.6%
	WMGr (%)				**22.1%**	**23.5%**
27	MLW (mg)		167.4			
	Gr (%)		-0.7%			
28	MLW (mg)		180.2			
	Gr (%)		7.6%			
29	MLW (mg)		190.8			
	Gr (%)		5.9%			
30	MLW (mg)		203.4			
	Gr (%)		6.6%			
31	MLW (mg)		205.8			
	Gr (%)		1.2%			
32	MLW (mg)		202.0			
	Gr (%)		-1.9%			
33	MLW (mg)		208.4			
	Gr (%)		3.2%			
34	MLW (mg)		220.3			
	Gr (%)		5.7%			
35	MLW (mg)		223.3			
	Gr (%)		1.3%			
36	MLW (mg)		221.3			
	Gr (%)		-0.9%			
	WMGr (%)		**23.4%**			

Table A13

Incubator 2 (I2), 20 °C, 8L/16D

Mean larval weight (MLW) in mg, growth rate (Gr) in % and weighted mean growth rate (WMGr) in % of every box during every week of data collection.

Week	**Term**	**n**									
		11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
0	MLW (mg)	1.9	1.6	1.3	1.8	1.7	1.9	1.7	2.0	1.3	1.2
1	MLW (mg)	3.2	2.6	2.1	3.0	2.6	3.2	2.7	3.2	2.1	1.8
	Gr (%)	65.7%	63.1%	57.9%	67.9%	55.5%	67.6%	66.1%	61.7%	59.7%	48.0%
2	MLW (mg)	3.7	3.2	2.8	3.7	3.3	4.1	3.9	4.4	2.6	2.1
	Gr (%)	16.8%	24.3%	33.9%	24.1%	27.6%	30.0%	40.7%	36.8%	20.4%	18.2%
3	MLW (mg)	5.3	4.5	3.4	4.9	4.2	5.2	4.5	5.4	3.5	2.9
	Gr (%)	44.1%	41.1%	23.5%	33.1%	25.7%	26.6%	15.4%	21.6%	35.1%	39.8%
4	MLW (mg)	6.6	5.6	4.5	5.9	5.4	6.5	6.2	7.0	4.1	3.4
	Gr (%)	23.6%	24.2%	29.6%	21.1%	29.1%	23.9%	39.4%	30.2%	18.9%	16.9%
5	MLW (mg)	8.5	7.5	5.6	8.3	6.9	8.5	7.2	9.0	5.5	4.7
	Gr (%)	30.3%	32.9%	26.5%	40.1%	26.9%	31.7%	16.1%	27.3%	32.8%	39.2%
6	MLW (mg)	10.4	9.2	7.0	9.9	8.6	10.4	9.5	11.6	6.5	6.2
	Gr (%)	21.7%	22.6%	23.2%	19.1%	24.8%	22.5%	31.3%	29.2%	19.1%	30.0%
7	MLW (mg)	14.1	12.9	9.6	13.5	10.9	13.6	11.5	15.1	8.7	8.1
	Gr (%)	35.9%	40.3%	38.7%	36.7%	27.2%	30.1%	21.1%	30.8%	34.5%	31.1%
8	MLW (mg)	18.1	15.9	12.2	16.2	13.5	17.0	14.6	18.7	10.5	10.0
	Gr (%)	28.4%	23.5%	26.6%	20.3%	23.9%	25.7%	27.0%	23.5%	20.2%	23.7%
9	MLW (mg)	22.5	20.4	15.5	21.4	16.0	20.8	17.9	24.6	13.2	12.3
	Gr (%)	24.0%	28.3%	27.1%	31.6%	18.4%	21.9%	22.9%	31.8%	26.0%	23.5%
10	MLW (mg)	32.6	27.0	20.8	28.3	23.3	31.0	23.7	31.6	18.0	16.7
	Gr (%)	44.8%	32.1%	34.2%	32.4%	44.9%	49.4%	32.1%	28.3%	36.0%	35.6%
11	MLW (mg)	41.5	36.3	28.2	35.9	28.1	37.3	30.2	42.9	24.3	21.7

	Gr (%)	27.5%	34.6%	35.2%	27.0%	20.8%	20.3%	27.7%	35.7%	35.2%	29.7%
12	MLW (mg)	55.0	47.8	38.4	49.1	40.9	55.6	42.5	54.9	32.1	30.4
	Gr (%)	32.4%	31.5%	36.3%	36.8%	45.6%	48.9%	40.6%	28.1%	32.1%	40.3%
13	MLW (mg)	71.4	60.5	49.5	55.4	48.6	62.3	49.8	67.5	41.9	39.1
	Gr (%)	29.8%	26.6%	29.1%	12.9%	18.8%	12.1%	17.1%	22.9%	30.4%	28.8%
14	MLW (mg)	81.9	76.9	64.0	72.5	66.6	82.1	65.8	82.6	54.2	50.7
	Gr (%)	14.7%	27.1%	29.2%	30.7%	37.0%	31.7%	32.1%	22.3%	29.3%	29.6%
15	MLW (mg)	101.1	90.6	82.3	79.5	79.1	94.2	75.5	92.1	65.9	68.6
	Gr (%)	23.6%	17.9%	28.5%	9.7%	18.8%	14.8%	14.8%	11.5%	21.7%	35.3%
16	MLW (mg)	110.4	104.2	96.6	85.6	95.3	104.8	89.6	95.6	81.5	83.0
	Gr (%)	9.1%	15.1%	17.4%	7.7%	20.4%	11.2%	18.7%	3.8%	23.6%	21.0%
17	MLW (mg)	112.0	113.4	114.4	102.0	111.1	118.2	96.9	108.0	92.1	90.6
	Gr (%)	1.5%	8.7%	18.5%	19.2%	16.5%	12.8%	8.1%	12.9%	12.9%	9.1%
18	MLW (mg)	115.5	115.7	115.1	102.2	111.3	115.9	100.9	108.2	97.8	97.8
	Gr (%)	3.1%	2.1%	0.6%	0.2%	0.2%	-1.9%	4.1%	0.2%	6.2%	8.0%
	WMGr (%)								**27.4%**		
19	MLW (mg)	102.0	133.7	123.5	89.2	118.4	121.1	100.6		102.9	96.9
	Gr (%)	-11.7%	15.6%	7.3%	-12.7%	6.4%	4.5%	-0.3%		5.2%	-0.9%
	WMGr (%)				**26.9%**						
20	MLW (mg)	100.6	126.5	128.8		127.9	126.2	99.5		104.0	92.4
	Gr (%)	-1.4%	-5.4%	4.3%		8.0%	4.2%	-1.1%		1.1%	-4.7%
	WMGr (%)		**28.3%**	**29.0%**			**27.5%**	**25.9%**			
21	MLW (mg)	98.1				124.5				106.4	88.4
	Gr (%)	-2.4%				-2.6%				2.3%	-4.3%
	WMGr (%)	**27.1%**				**25.9%**					
22	MLW (mg)									116.6	92.2
	Gr (%)									9.6%	4.2%
	WMGr (%)										
23	MLW (mg)									113.3	60.7
	Gr (%)									-2.9%	-34.1%
	WMGr (%)									**25.9%**	**26.2%**

Table A14

Incubator 3 (I3), 20 °C, 24D

Mean larval weight (MLW) in mg, growth rate (Gr) in % and weighted mean growth rate (WMGr) in % of every box during every week of data collection.

Week	**Term**	**n**									
		21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
0	MLW (mg)	1.2	1.3	1.6	1.3	1.8	1.7	1.6	1.8	1.7	2.0
1	MLW (mg)	1.9	1.9	2.6	2.1	2.9	2.8	2.6	2.6	2.6	3.1
	Gr (%)	64.8%	50.9%	60.2%	66.8%	61.9%	60.8%	64.7%	43.6%	54.5%	58.3%
2	MLW (mg)	2.6	2.5	3.1	2.7	3.3	3.4	3.3	3.2	3.0	4.1
	Gr (%)	34.1%	28.1%	18.6%	30.4%	17.1%	22.0%	25.7%	24.2%	17.8%	29.6%
3	MLW (mg)	3.3	3.1	4.2	3.3	4.4	4.5	4.4	4.1	4.2	5.2
	Gr (%)	29.1%	27.1%	34.4%	20.9%	32.8%	33.3%	31.3%	26.1%	38.7%	28.2%
4	MLW (mg)	4.0	3.8	5.0	4.4	5.4	5.6	5.3	5.2	5.2	6.9
	Gr (%)	20.6%	23.0%	19.8%	32.1%	21.7%	24.4%	22.5%	26.7%	24.4%	32.0%
5	MLW (mg)	5.4	4.9	6.4	5.7	6.7	7.5	7.2	6.4	7.2	8.8
	Gr (%)	33.0%	28.2%	28.4%	28.9%	23.8%	33.9%	35.7%	23.8%	38.3%	28.1%
6	MLW (mg)	6.1	5.7	7.7	7.2	8.6	9.1	8.4	8.4	9.0	11.7
	Gr (%)	14.0%	16.7%	19.7%	26.5%	28.4%	22.7%	16.2%	31.0%	24.7%	32.6%
7	MLW (mg)	8.8	8.1	10.0	9.4	10.4	10.9	12.2	9.9	12.0	14.8
	Gr (%)	44.9%	40.7%	30.3%	31.4%	20.9%	19.1%	45.1%	18.1%	33.7%	26.5%
8	MLW (mg)	10.6	9.3	12.5	11.4	13.2	13.7	14.0	13.2	15.2	19.3
	Gr (%)	19.4%	14.8%	25.2%	21.8%	27.3%	25.7%	14.6%	33.8%	26.7%	30.3%
9	MLW (mg)	14.0	11.8	14.8	15.0	15.9	16.6	18.7	14.7	18.6	24.1

	Gr (%)	32.2%	27.2%	18.8%	30.8%	20.4%	21.6%	33.8%	10.9%	21.8%	25.1%
10	MLW (mg)	18.1	15.9	18.8	18.9	20.4	21.3	23.3	20.7	26.0	30.9
	Gr (%)	29.6%	35.0%	26.6%	26.2%	28.3%	28.2%	24.1%	40.8%	39.9%	28.2%
11	MLW (mg)	22.4	17.9	23.1	24.3	25.4	27.1	28.9	23.8	31.0	39.7
	Gr (%)	23.7%	12.4%	23.0%	28.6%	24.2%	26.8%	24.3%	15.3%	19.2%	28.5%
12	MLW (mg)	31.0	26.7	31.4	32.0	33.4	35.5	40.2	33.1	43.3	51.4
	Gr (%)	38.5%	49.1%	35.8%	31.6%	31.7%	31.3%	39.1%	38.9%	39.9%	29.5%
13	MLW (mg)	37.2	29.6	37.8	40.1	39.9	43.2	46.1	39.1	50.3	62.7
	Gr (%)	19.9%	10.8%	20.6%	25.3%	19.5%	21.7%	14.6%	18.1%	16.1%	22.0%
14	MLW (mg)	51.2	42.3	49.1	51.7	52.7	53.7	61.0	48.7	66.2	77.2
	Gr (%)	37.8%	43.1%	29.7%	29.1%	31.9%	24.3%	32.3%	24.5%	31.7%	23.1%
15	MLW (mg)	58.9	50.6	62.0	64.5	61.3	65.6	72.2	62.1	77.3	94.0
	Gr (%)	15.0%	19.5%	26.5%	24.7%	16.4%	22.1%	18.5%	27.5%	16.8%	21.9%
16	MLW (mg)	75.5	60.5	75.4	75.5	75.9	77.9	84.1	70.0	97.1	106.8
	Gr (%)	28.1%	19.5%	21.5%	17.1%	23.8%	18.8%	16.5%	12.7%	25.5%	13.6%
17	MLW (mg)	90.3	80.2	87.9	93.4	92.5	92.6	102.5	86.4	106.6	120.9
	Gr (%)	19.7%	32.6%	16.6%	23.7%	21.9%	18.8%	21.8%	23.4%	9.9%	13.2%
18	MLW (mg)	94.3	84.6	93.7	100.8	100.4	104.9	110.0	94.6	120.7	126.7
	Gr (%)	4.4%	5.5%	6.6%	7.9%	8.5%	13.3%	7.4%	9.5%	13.2%	4.8%
19	MLW (mg)	107.1	103.1	105.0	106.5	115.8	115.8	120.2	99.3	128.1	131.6
	Gr (%)	13.6%	21.9%	12.1%	5.7%	15.3%	10.3%	9.2%	5.0%	6.2%	3.9%
20	MLW (mg)	106.9	110.1	99.7	121.0	125.5	127.5	137.2	108.1	125.9	132.7
	Gr (%)	-0.2%	6.9%	-5.1%	13.6%	8.4%	10.1%	14.2%	8.8%	-1.7%	0.8%
	WMGr (%)									**26.2%**	
21	MLW (mg)	111.1	102.0	106.0	122.4	127.3	134.0	136.9	104.7		125.3
	Gr (%)	4.0%	-7.4%	6.3%	1.2%	1.5%	5.1%	-0.3%	-3.2%		-5.6%
	WMGr (%)	**26.5%**									**25.5%**
22	MLW (mg)		108.8	114.9	134.0	132.6	142.8	129.3	102.5		
	Gr (%)		6.6%	8.4%	9.4%	4.2%	6.6%	-5.5%	-2.0%		
	WMGr (%)							**25.2%**			
23	MLW (mg)		129.1	113.0	129.7	131.1	131.6		106.5		
	Gr (%)		18.6%	-1.7%	-3.2%	-1.2%	-7.9%		3.9%		
	WMGr (%)			**24.3%**	**25.9%**	**23.7%**	**24.2%**				
24	MLW (mg)		126.4						121.6		
	Gr (%)		-2.0%						14.2%		
	WMGr (%)										
25	MLW (mg)		146.1						116.7		
	Gr (%)		15.6%						-4.1%		
	WMGr (%)										
26	MLW (mg)		173.4						125.9		
	Gr (%)		18.7%						7.9%		
	WMGr (%)										
27	MLW (mg)		168.0						147.3		
	Gr (%)		-3.1%						17.0%		
	WMGr (%)		**24.1%**								
28	MLW (mg)								152.2		
	Gr (%)								3.3%		
	WMGr (%)								**22.2%**		

Table A15

Incubator 4 (I4), 25 °C, 16L/8D

Mean larval weight (MLW) in mg, growth rate (Gr) in % and weighted mean growth rate (WMGr) in % of every box during every week of data collection.

Week	Term	n									
		31	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**
0	MLW (mg)	1.4	1.6	1.5	1.6	1.7	1.6	1.6	1.3	1.9	1.5
1	MLW (mg)	2.0	2.3	2.1	2.2	2.5	2.1	2.2	1.9	2.7	2.3
	Gr (%)	41.5%	40.5%	39.2%	36.2%	46.8%	30.4%	44.3%	47.7%	39.4%	51.8%
2	MLW (mg)	3.1	3.5	3.3	3.1	4.0	3.2	3.3	3.2	4.4	4.0
	Gr (%)	57.3%	52.8%	57.1%	41.3%	61.8%	52.5%	46.6%	73.4%	65.3%	70.0%
3	MLW (mg)	4.9	5.6	5.2	5.3	6.5	4.6	5.7	5.1	7.3	6.5
	Gr (%)	55.9%	61.9%	59.3%	71.2%	62.3%	43.0%	71.7%	58.4%	64.4%	64.7%
4	MLW (mg)	6.8	8.2	7.7	7.6	8.7	6.4	8.5	7.1	10.5	9.4
	Gr (%)	39.1%	45.6%	47.2%	43.8%	35.4%	39.7%	51.1%	40.5%	44.8%	44.6%
5	MLW (mg)	9.5	11.1	10.5	11.1	12.3	8.2	10.3	9.1	13.2	12.8
	Gr (%)	40.2%	35.2%	37.3%	45.6%	40.5%	26.9%	20.8%	27.7%	26.0%	35.2%
6	MLW (mg)	13.9	16.2	16.1	16.3	18.8	11.2	15.5	14.6	20.6	20.2
	Gr (%)	46.7%	47.0%	52.6%	47.1%	52.8%	36.4%	50.4%	60.0%	55.5%	58.2%
7	MLW (mg)	21.0	23.1	24.1	25.1	28.5	16.4	26.5	23.1	31.7	31.2
	Gr (%)	50.8%	42.2%	50.3%	53.6%	51.8%	46.8%	70.7%	58.4%	53.8%	54.7%
8	MLW (mg)	28.9	31.7	35.1	34.3	39.7	23.3	36.0	30.4	41.0	44.1
	Gr (%)	37.6%	37.2%	45.6%	36.9%	39.5%	42.0%	35.7%	31.5%	29.3%	41.1%
9	MLW (mg)	43.0	43.9	50.0	45.9	55.2	30.8	47.2	43.8	56.3	61.4
	Gr (%)	48.7%	38.6%	42.2%	33.6%	38.9%	32.2%	31.2%	43.9%	37.4%	39.3%
10	MLW (mg)	60.0	63.0	65.0	63.0	75.0	43.0	63.0	59.0	73.0	80.0
	Gr (%)	39.7%	43.4%	30.1%	37.3%	35.9%	39.8%	33.5%	34.7%	29.6%	30.2%
11	MLW (mg)	78.0	86.3	86.9	81.0	96.8	58.4	83.2	77.6	92.4	106.6
	Gr (%)	29.9%	37.0%	33.8%	28.6%	29.0%	35.8%	32.1%	31.4%	26.6%	33.3%
12	MLW (mg)	94.2	104.1	107.6	97.7	115.4	70.4	107.8	93.7	109.6	124.4
	Gr (%)	20.8%	20.7%	23.8%	20.6%	19.3%	20.5%	29.6%	20.9%	18.6%	16.7%
13	MLW (mg)	113.1	115.7	119.9	102.8	127.1	83.8	111.8	115.6	130.7	144.7
	Gr (%)	20.1%	11.1%	11.4%	5.3%	10.1%	19.0%	3.7%	23.3%	19.2%	16.3%
14	MLW (mg)	122.2	128.3	128.0	110.1	135.8	86.0	120.2	120.4	138.3	149.9
	Gr (%)	8.1%	10.8%	6.7%	7.0%	6.9%	2.6%	7.5%	4.1%	5.8%	3.6%
15	MLW (mg)	132.5	137.9	139.6	104.7	140.3	85.7	121.9	135.9	158.3	168.3
	Gr (%)	8.5%	7.5%	9.1%	-4.9%	3.3%	-0.4%	1.4%	12.9%	14.5%	12.3%
16	MLW (mg)	140.7	140.9	142.7	117.3	152.6	90.9	129.5	133.3	156.5	167.6
	Gr (%)	6.2%	2.2%	2.2%	12.1%	8.8%	6.2%	6.3%	-1.9%	-1.1%	-0.4%
	WMGr (%)			**37.8%**							**38.8%**
17	MLW (mg)	121.4	136.9		117.0	156.8	101.6	143.2	120.3	173.2	
	Gr (%)	**-13.7%**	-2.8%		-0.3%	2.8%	11.7%	10.5%	-9.7%	10.7%	
	WMGr (%)	36.1%				**36.4%**		**37.9%**			
18	MLW (mg)		137.2		106.7		106.3		102.6	174.3	
	Gr (%)		0.2%		-8.8%		4.7%		-14.7%	0.6%	
	WMGr (%)		**35.8%**						**35.9%**		
19	MLW (mg)				120.5		115.7			193.6	
	Gr (%)				13.0%		8.8%			11.1%	
	WMGr (%)										
20	MLW (mg)				130.1		108.3			189.2	
	Gr (%)				8.0%		-6.4%			-2.3%	
	WMGr (%)									**31.8%**	
21	MLW (mg)				126.1		82.5				
	Gr (%)				-3.1%		-23.9%				
	WMGr (%)										
22	MLW (mg)				149.8		61.9				
	Gr (%)				18.8%		-24.9%				
	WMGr (%)										
23	MLW (mg)				138.8		71.5				

	Gr (%)			-7.4%		15.5%				
	WMGr (%)			**32.4%**						
24	MLW (mg)					84.2				
	Gr (%)					17.7%				
	WMGr (%)									
25	MLW (mg)					92.9				
	Gr (%)					10.3%				
	WMGr (%)									
26	MLW (mg)					106.8				
	Gr (%)					15.0%				
	WMGr (%)									
27	MLW (mg)					101.7				
	Gr (%)					-4.7%				
	WMGr (%)					**27.9%**				

Table A16

Incubator 5 (I5), 25 °C, 8L/16D

Mean larval weight (MLW) in mg, growth rate (Gr) in % and weighted mean growth rate (WMGr) in % of every box during every week of data collection.

Week	**Term**	**n**									
		41	**42**	**43**	**44**	**45**	**46**	**48**	**48**	**49**	**50**
0	MLW (mg)	1.3	1.6	1.5	1.3	1.5	1.6	1.4	1.9	1.5	1.6
1	MLW (mg)	2.0	1.9	2.2	2.0	2.0	2.6	2.1	2.5	2.0	2.2
	Gr (%)	48.0%	20.6%	48.4%	51.4%	31.4%	60.7%	46.9%	34.9%	32.1%	42.7%
2	MLW (mg)	2.9	2.9	3.4	2.9	3.0	3.7	2.9	3.6	3.1	3.4
	Gr (%)	46.8%	51.0%	50.1%	47.9%	51.3%	41.5%	37.6%	40.2%	55.0%	55.5%
3	MLW (mg)	4.4	4.5	4.5	4.7	4.3	5.1	4.7	5.1	4.7	5.1
	Gr (%)	54.9%	52.6%	34.6%	60.8%	44.8%	36.5%	63.2%	44.1%	50.7%	47.4%
4	MLW (mg)	6.5	6.7	6.4	6.6	5.6	7.2	7.3	7.4	6.8	7.0
	Gr (%)	47.2%	49.6%	41.1%	41.4%	30.0%	40.7%	56.0%	43.5%	43.0%	38.6%
5	MLW (mg)	9.2	9.8	8.6	10.0	7.4	10.5	9.7	9.5	8.3	9.8
	Gr (%)	41.1%	46.4%	35.2%	50.9%	31.3%	45.6%	32.3%	29.3%	22.1%	39.8%
6	MLW (mg)	13.3	13.1	11.9	13.6	10.4	14.4	13.7	12.5	12.5	14.6
	Gr (%)	44.3%	32.8%	38.3%	35.9%	41.0%	37.5%	41.6%	31.9%	51.7%	48.1%
7	MLW (mg)	19.3	18.5	17.4	20.5	13.6	19.9	19.5	18.5	17.1	19.4
	Gr (%)	45.2%	41.7%	45.3%	50.4%	30.2%	38.4%	41.8%	47.7%	36.5%	33.0%
8	MLW (mg)	27.2	28.4	24.0	31.2	20.5	28.1	26.9	26.4	23.2	28.0
	Gr (%)	40.5%	53.4%	38.2%	52.3%	50.9%	41.1%	37.8%	42.4%	36.0%	44.7%
9	MLW (mg)	36.7	40.0	32.1	40.2	26.2	37.0	35.8	34.9	32.9	37.4
	Gr (%)	35.3%	40.8%	34.0%	29.0%	28.0%	31.8%	33.1%	32.4%	41.5%	33.4%
10	MLW (mg)	50.0	55.0	44.0	56.0	37.0	51.0	49.0	48.0	45.0	49.0
	Gr (%)	36.1%	37.7%	36.9%	39.3%	41.2%	37.8%	37.1%	37.4%	36.8%	30.9%
11	MLW (mg)	65.7	72.4	57.3	75.1	51.0	66.1	62.7	62.6	58.1	65.1
	Gr (%)	31.3%	31.6%	30.2%	34.1%	37.9%	29.5%	28.0%	30.3%	29.2%	32.8%
12	MLW (mg)	82.5	86.7	72.0	88.9	61.4	78.1	74.5	84.3	74.2	78.6
	Gr (%)	25.7%	19.8%	25.7%	18.4%	20.4%	18.3%	18.8%	34.7%	27.6%	20.8%
13	MLW (mg)	97.4	107.1	89.6	110.5	80.4	97.1	90.3	97.2	88.8	94.7
	Gr (%)	18.0%	23.4%	24.4%	24.4%	30.8%	24.4%	21.2%	15.4%	19.7%	20.5%
14	MLW (mg)	109.2	118.2	100.7	113.5	89.7	106.4	95.1	111.0	103.1	107.0
	Gr (%)	12.2%	10.4%	12.5%	2.7%	11.7%	9.5%	5.3%	14.2%	16.1%	12.9%
15	MLW (mg)	115.2	130.6	117.0	130.2	101.5	113.6	99.7	113.4	108.8	99.0
	Gr (%)	5.5%	10.5%	16.1%	14.8%	13.1%	6.8%	4.8%	2.2%	5.5%	-7.5%
16	MLW (mg)	123.6	131.9	116.7	130.6	107.9	111.7	98.4	122.3	118.2	91.0
	Gr (%)	7.3%	1.0%	-0.3%	0.3%	6.3%	-1.6%	-1.3%	7.8%	8.6%	-8.1%
	WMGr (%)		**34.6%**		**37.6%**						
17	MLW (mg)	119.4		128.6		104.2	119.1	91.0	115.2	115.4	81.9

	Gr (%)	-3.4%	10.3%	-3.4%	6.6%	-7.5%	-5.7%	-2.4%	-10.1%
	WMGr (%)								
18	MLW (mg)	130.7	126.7	95.0	117.8	75.1	125.0	110.8	94.2
	Gr (%)	9.4%	-1.5%	-8.9%	-1.0%	-17.5%	8.5%	-4.0%	15.1%
	WMGr (%)	**34.6%**			**31.6%**				
19	MLW (mg)		137.3	94.6		77.0	136.2	116.9	95.7
	Gr (%)		8.3%	-0.4%		2.5%	9.0%	5.5%	1.6%
	WMGr (%)								
20	MLW (mg)		142.5	93.7		78.2	137.1	114.2	87.6
	Gr (%)		3.8%	-0.9%		1.6%	0.7%	-2.3%	-8.4%
	WMGr (%)		**30.6%**					**31.2%**	**32.0%**
21	MLW (mg)			94.3		76.3	154.8		
	Gr (%)			0.7%		-2.4%	12.9%		
	WMGr (%)						**30.7%**		
22	MLW (mg)			96.0		75.6			
	Gr (%)			1.8%		-0.9%			
	WMGr (%)					**31.2%**			
23	MLW (mg)			103.7					
	Gr (%)			8.0%					
	WMGr (%)								
24	MLW (mg)			117.8					
	Gr (%)			13.6%					
	WMGr (%)			**29.0%**					

Table A17

Incubator 6 (I6), 25 °C, 24D

Mean larval weight (MLW) in mg, growth rate (Gr) in % and weighted mean growth rate (WMGr) in % of every box during every week of data collection.

Week	**Term**	**n**									
		51	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**
0	MLW (mg)	2.1	1.6	1.6	2.1	1.6	2.0	2.0	2.0	1.3	1.9
1	MLW (mg)	3.3	2.5	2.5	3.2	2.5	3.1	3.1	3.1	1.7	2.9
	Gr (%)	56.9%	57.1%	56.0%	51.3%	56.8%	55.3%	56.3%	57.6%	37.7%	54.1%
2	MLW (mg)	5.2	4.0	3.9	4.2	3.8	4.7	4.5	5.0	2.6	4.5
	Gr (%)	55.0%	57.4%	56.7%	32.5%	52.4%	50.6%	48.3%	61.2%	51.9%	53.4%
3	MLW (mg)	7.9	6.2	6.0	6.9	5.8	7.0	7.3	8.0	4.0	6.8
	Gr (%)	53.8%	55.0%	52.5%	63.3%	52.9%	46.9%	62.1%	57.7%	51.4%	50.2%
4	MLW (mg)	12.1	9.5	8.6	10.0	8.3	9.5	10.6	11.9	6.4	9.8
	Gr (%)	52.7%	53.8%	42.7%	45.2%	42.4%	36.4%	44.4%	49.7%	61.1%	45.4%
5	MLW (mg)	16.4	13.3	12.0	15.3	12.7	15.8	13.9	16.7	9.2	14.4
	Gr (%)	35.4%	39.5%	40.1%	53.2%	52.3%	66.9%	31.0%	40.1%	42.8%	47.1%
6	MLW (mg)	24.3	19.2	18.1	21.8	17.8	23.5	21.5	24.2	12.3	21.5
	Gr (%)	48.2%	44.6%	50.6%	42.2%	40.4%	48.1%	54.5%	45.3%	34.1%	48.6%
7	MLW (mg)	39.8	31.5	28.6	33.0	27.4	34.1	33.0	38.6	19.2	31.8
	Gr (%)	63.7%	63.5%	58.1%	51.5%	54.1%	45.4%	53.9%	59.1%	56.2%	48.3%
8	MLW (mg)	58.3	47.5	45.1	52.8	42.5	51.7	47.1	58.4	32.7	49.7
	Gr (%)	46.5%	50.9%	57.6%	59.9%	55.0%	51.6%	42.6%	51.3%	70.2%	56.2%
9	MLW (mg)	77.3	67.4	62.7	71.7	57.6	79.2	73.2	79.7	50.4	72.2
	Gr (%)	32.5%	42.0%	39.0%	35.7%	35.5%	53.1%	55.5%	36.6%	53.9%	45.3%
10	MLW (mg)	100.0	90.0	90.0	93.0	77.0	102.0	94.0	104.0	72.0	98.0
	Gr (%)	29.4%	33.4%	43.5%	29.8%	33.7%	28.8%	28.3%	30.4%	42.9%	35.7%
11	MLW (mg)	129.4	119.3	118.9	115.3	98.9	128.4	117.9	128.9	95.7	124.5
	Gr (%)	29.4%	32.5%	32.1%	23.9%	28.5%	25.9%	25.5%	23.9%	33.0%	27.0%
12	MLW (mg)	143.4	135.8	140.8	123.0	110.1	157.8	133.6	145.3	119.4	138.4
	Gr (%)	10.8%	13.8%	18.4%	6.8%	11.3%	22.9%	13.3%	12.7%	24.8%	11.1%
13	MLW (mg)	158.4	133.7	154.8	136.9	117.9	147.5	136.9	153.2	135.3	155.8

	Gr (%)	10.5%	-1.6%	9.9%	11.3%	7.1%	-6.5%	2.4%	5.4%	13.3%	12.6%
14	MLW (mg)	155.4	130.1	138.1	132.1	121.1	167.2	122.2	152.4	142.8	155.7
	Gr (%)	-1.9%	-2.7%	-10.7%	-3.5%	2.7%	13.4%	-10.7%	-0.5%	5.5%	0.0%
	WMGr (%)		**43.5%**			**41.2%**	**43.4%**	**39.7%**	**41.9%**		**41.0%**
15	MLW (mg)	146.7		111.6	141.7					142.3	
	Gr (%)	-5.6%		-19.2%	7.2%					-0.3%	
	WMGr (%)			**41.8%**						**41.0%**	
16	MLW (mg)	165.8			150.8						
	Gr (%)	13.0%			6.4%						
	WMGr (%)	**39.8%**			**38.4%**						

Table A18

Incubator 7 (I7), 30 °C, 16L/8D

Mean larval weight (MLW) in mg, growth rate (Gr) in % and weighted mean growth rate (WMGr) in % of every box during every week of data collection.

Week	**Term**	**n**									
		61	**62**	**63**	**64**	**65**	**66**	**67**	**68**	**69**	**70**
0	MLW (mg)	2.1	1.3	1.5	1.1	1.3	1.6	1.6	1.6	1.1	1.2
1	MLW (mg)	3.7	2.0	2.5	1.8	2.2	2.6	2.6	2.8	2.0	2.1
	Gr (%)	76.3%	61.3%	61.7%	63.5%	63.7%	69.1%	59.6%	73.3%	80.3%	65.8%
2	MLW (mg)	5.8	3.3	3.9	2.7	3.3	4.0	4.0	4.5	3.2	3.0
	Gr (%)	58.2%	60.3%	58.0%	47.1%	54.1%	50.4%	54.5%	61.6%	60.2%	46.3%
3	MLW (mg)	9.3	5.1	6.2	4.2	5.0	6.7	7.2	7.1	5.1	4.9
	Gr (%)	59.7%	54.6%	59.8%	54.5%	50.5%	68.8%	81.8%	55.4%	61.9%	61.4%
4	MLW (mg)	13.6	7.2	9.4	6.2	7.2	9.8	11.2	10.3	8.0	7.1
	Gr (%)	46.2%	41.5%	51.1%	47.5%	44.1%	45.7%	55.9%	45.5%	55.4%	45.5%
5	MLW (mg)	18.6	10.6	13.8	9.2	11.0	13.5	15.6	14.0	11.6	10.3
	Gr (%)	37.2%	47.9%	47.2%	48.2%	52.1%	38.3%	38.7%	36.2%	45.0%	45.1%
6	MLW (mg)	25.3	15.1	19.4	13.0	16.2	18.0	22.4	20.3	17.4	15.4
	Gr (%)	35.8%	42.7%	40.0%	41.6%	47.5%	33.6%	43.8%	45.0%	50.5%	50.4%
7	MLW (mg)	39.2	23.7	29.5	20.6	25.2	27.6	36.6	32.3	29.4	26.0
	Gr (%)	55.1%	56.7%	52.1%	59.1%	55.4%	53.1%	63.4%	59.0%	68.9%	68.3%
8	MLW (mg)	60.6	36.1	44.7	33.8	39.1	41.8	57.9	47.7	46.5	39.0
	Gr (%)	54.7%	52.4%	51.4%	63.6%	54.9%	51.4%	58.2%	47.6%	58.3%	50.3%
9	MLW (mg)	85.7	53.4	70.0	51.8	56.9	59.1	79.1	68.3	71.3	59.7
	Gr (%)	41.3%	48.0%	56.7%	53.5%	45.6%	41.3%	36.7%	43.2%	53.2%	53.2%
10	MLW (mg)	113.8	75.5	95.6	77.0	80.5	83.2	110.0	93.0	97.3	79.7
	Gr (%)	32.8%	41.2%	36.7%	48.6%	41.5%	40.9%	39.1%	36.2%	36.6%	33.4%
11	MLW (mg)	135.9	105.3	120.5	103.4	111.7	112.9	147.3	115.1	125.5	108.8
	Gr (%)	19.4%	39.6%	26.0%	34.3%	38.8%	35.6%	33.9%	23.8%	28.9%	36.5%
12	MLW (mg)	151.1	118.5	140.6	129.9	121.9	130.0	160.6	130.1	143.2	121.1
	Gr (%)	11.2%	12.5%	16.7%	25.6%	9.1%	15.2%	9.0%	13.0%	14.1%	11.3%
13	MLW (mg)	160.6	129.8	146.9	137.9	140.2	143.0	175.4	139.0	155.2	147.3
	Gr (%)	6.3%	9.5%	4.5%	6.2%	15.0%	10.0%	9.2%	6.8%	8.4%	21.6%
14	MLW (mg)	167.9	139.6	160.2	157.6	151.6	147.3	181.2	146.4	161.3	154.2
	Gr (%)	4.5%	7.6%	9.1%	14.3%	8.2%	3.0%	3.3%	5.4%	3.9%	4.7%
15	MLW (mg)	186.5	149.8	165.6	153.4	149.6	155.9	183.2	159.8	176.6	157.1
	Gr (%)	11.1%	7.3%	3.3%	-2.6%	-1.4%	5.8%	1.1%	9.1%	9.5%	1.9%
16	MLW (mg)	181.0	152.0	169.3	159.8	150.2	162.7	187.5	171.7	174.3	164.0
	Gr (%)	-2.9%	1.5%	2.2%	4.2%	0.4%	4.4%	2.4%	7.5%	-1.3%	4.4%
	WMGr (%)									**44.2%**	
17	MLW (mg)	185.0	154.7	153.1	148.9	154.7	163.8	189.6	174.7		153.6
	Gr (%)	2.2%	1.7%	-9.5%	-6.8%	3.0%	0.7%	1.1%	1.7%		-6.4%
	WMGr (%)				**41.1%**				**41.2%**		
18	MLW (mg)	177.1	152.2	153.3		162.3	163.0	192.3			154.5
	Gr (%)	-4.3%	-1.6%	0.1%		4.9%	-0.5%	1.4%			0.6%

	WMGr (%)								
19	MLW (mg)	183.5	164.8	139.3	165.6	182.9	194.8		157.1
	Gr (%)	3.6%	8.3%	-9.2%	2.0%	12.2%	1.3%		1.6%
	WMGr (%)					**38.0%**			
20	MLW (mg)	200.9	158.8	123.8	173.0		198.8		159.1
	Gr (%)	9.4%	-3.6%	-11.1%	4.5%		2.1%		1.3%
	WMGr (%)			**38.4%**					
21	MLW (mg)	190.7	163.3		177.0		209.3		162.3
	Gr (%)	-5.1%	2.8%		2.3%		5.2%		2.0%
	WMGr (%)	**37.9%**			**37.8%**		**37.8%**		
22	MLW (mg)		170.5						145.8
	Gr (%)		4.4%						-10.1%
	WMGr (%)		**36.1%**						
23	MLW (mg)								165.3
	Gr (%)								13.4%
	WMGr (%)								**35.3%**

Table A19

Incubator 8 (I8), 30 °C, 8L/16D

Mean larval weight (MLW) in mg, growth rate (Gr) in % and weighted mean growth rate (WMGr) in % of every box during every week of data collection.

Week	**Term**	**n**									
		71	**72**	**73**	**74**	**75**	**76**	**77**	**78**	**79**	**80**
0	MLW (mg)	1.5	1.3	1.8	1.7	2.2	1.8	1.9	1.5	2.0	1.9
1	MLW (mg)	2.3	2.0	2.9	2.7	3.5	2.8	3.0	2.5	3.3	3.0
	Gr (%)	59.0%	50.7%	58.7%	56.7%	59.4%	51.7%	58.6%	59.9%	65.5%	55.5%
2	MLW (mg)	3.7	3.0	4.5	4.3	5.6	3.9	4.3	3.9	5.3	4.6
	Gr (%)	57.7%	52.0%	58.0%	59.2%	58.9%	42.3%	43.2%	56.6%	62.3%	54.8%
3	MLW (mg)	5.6	4.5	6.8	6.5	8.6	6.3	7.0	6.0	8.0	7.0
	Gr (%)	50.8%	48.2%	51.0%	50.9%	52.3%	59.6%	63.1%	54.9%	51.3%	52.7%
4	MLW (mg)	8.0	6.7	9.8	8.8	11.6	8.8	10.0	9.1	11.4	10.1
	Gr (%)	44.2%	48.1%	43.7%	33.9%	35.7%	39.7%	41.4%	52.5%	41.6%	43.8%
5	MLW (mg)	12.5	9.1	14.4	13.1	17.7	12.2	13.9	13.5	17.1	13.9
	Gr (%)	55.3%	35.4%	47.3%	49.8%	52.3%	39.2%	39.2%	47.7%	49.9%	38.1%
6	MLW (mg)	18.4	13.7	20.1	19.1	24.8	17.2	19.7	19.5	25.6	20.5
	Gr (%)	47.2%	50.7%	39.4%	45.3%	39.8%	40.7%	42.1%	44.8%	50.0%	47.6%
7	MLW (mg)	29.1	19.6	31.9	30.7	37.1	26.6	31.0	31.8	39.9	32.5
	Gr (%)	58.4%	43.7%	58.7%	60.8%	49.7%	54.6%	57.2%	62.9%	55.8%	58.6%
8	MLW (mg)	44.1	30.0	48.3	48.0	52.6	40.1	45.6	48.7	55.3	47.6
	Gr (%)	51.5%	53.0%	51.8%	56.4%	42.0%	50.6%	47.0%	53.3%	38.8%	46.3%
9	MLW (mg)	63.1	46.7	69.3	71.7	70.8	55.3	63.5	71.4	80.0	67.2
	Gr (%)	43.1%	55.5%	43.3%	49.3%	34.5%	37.9%	39.4%	46.6%	44.7%	41.2%
10	MLW (mg)	88.8	65.4	95.6	93.2	95.0	80.0	88.4	98.4	106.7	94.9
	Gr (%)	40.6%	40.1%	37.9%	30.0%	34.3%	44.6%	39.2%	37.8%	33.3%	41.2%
11	MLW (mg)	109.7	90.1	128.6	125.8	111.9	101.2	108.2	122.4	125.9	115.9
	Gr (%)	23.5%	37.8%	34.6%	34.9%	17.7%	26.5%	22.4%	24.4%	18.0%	22.1%
12	MLW (mg)	123.9	98.6	145.0	142.8	124.6	122.3	127.4	141.5	145.8	135.0
	Gr (%)	13.0%	9.4%	12.8%	13.6%	11.4%	20.9%	17.7%	15.5%	15.8%	16.5%
13	MLW (mg)	127.8	123.6	156.8	150.7	134.8	136.2	138.9	144.8	148.1	139.7
	Gr (%)	3.1%	25.4%	8.1%	5.5%	8.1%	11.4%	9.0%	2.4%	1.5%	3.5%
14	MLW (mg)	139.2	127.4	164.3	164.4	133.5	150.3	147.6	157.7	152.5	145.1
	Gr (%)	8.9%	3.1%	4.8%	9.1%	-0.9%	10.3%	6.3%	8.9%	3.0%	3.9%
15	MLW (mg)	133.5	144.0	173.7	168.1	132.2	156.7	155.1	140.7	161.6	144.3
	Gr (%)	-4.0%	13.0%	5.7%	2.3%	-1.0%	4.2%	5.1%	-10.8%	5.9%	-0.5%
	WMGr (%)					**36.7%**			**41.8%**		
16	MLW (mg)	127.2	134.9	189.7	184.7		160.2	145.4		144.1	138.8

	Gr (%)	-4.8%	-6.3%	9.2%	9.9%	2.3%	-6.2%	-10.8%	-3.9%
	WMGr (%)							**39.8%**	**37.9%**
17	MLW (mg)	139.4	142.4	190.5	160.8	159.7	150.8		
	Gr (%)	9.6%	5.5%	0.4%	-13.0%	-0.3%	3.7%		
	WMGr (%)	**40.5%**		**40.3%**	**38.6%**				
18	MLW (mg)		124.4			160.3	149.9		
	Gr (%)		-12.6%			0.4%	-0.7%		
	WMGr (%)						**37.3%**		
19	MLW (mg)		132.8			176.1			
	Gr (%)		6.7%			9.8%			
	WMGr (%)								
20	MLW (mg)		134.7			191.4			
	Gr (%)		1.5%			8.7%			
	WMGr (%)								
21	MLW (mg)		135.4			200.1			
	Gr (%)		0.5%			4.5%			
	WMGr (%)								
22	MLW (mg)		133.9			209.9			
	Gr (%)		-1.1%			4.9%			
	WMGr (%)		**34.3%**						
23	MLW (mg)					194.3			
	Gr (%)					-7.4%			
	WMGr (%)					**30.0%**			

Table A20

Incubator 9 (I9), 30 °C, 24D

Mean larval weight (MLW) in mg, growth rate (Gr) in % and weighted mean growth rate (WMGr) in % of every box during every week of data collection.

Week	**Term**	**n**									
		81	**82**	**83**	**84**	**85**	**86**	**87**	**88**	**89**	**90**
0	MLW (mg)	0.9	0.8	1.0	1.3	1.6	1.3	1.4	1.1	1.6	1.1
1	MLW (mg)	1.5	1.5	1.6	2.1	2.6	2.0	2.2	1.7	2.4	1.7
	Gr (%)	60.9%	92.1%	57.4%	59.8%	65.8%	48.3%	58.9%	49.5%	48.7%	45.1%
2	MLW (mg)	2.3	2.4	2.6	3.2	4.3	3.3	3.3	2.7	4.0	2.7
	Gr (%)	56.2%	62.3%	60.8%	53.3%	63.6%	66.2%	49.3%	56.4%	64.5%	63.4%
3	MLW (mg)	3.7	4.0	4.0	5.3	6.8	5.2	5.6	4.1	6.2	4.3
	Gr (%)	57.8%	67.8%	57.9%	64.9%	56.9%	57.8%	69.4%	53.5%	57.1%	58.9%
4	MLW (mg)	5.9	6.5	6.2	8.3	9.8	7.6	8.5	6.4	9.8	6.9
	Gr (%)	58.5%	62.3%	55.0%	56.4%	44.3%	45.8%	50.5%	55.1%	57.7%	60.0%
5	MLW (mg)	8.5	9.0	9.0	13.0	14.8	11.6	11.5	9.4	14.4	9.7
	Gr (%)	44.4%	38.2%	44.8%	56.2%	51.1%	53.5%	36.0%	46.6%	46.4%	41.0%
6	MLW (mg)	12.9	13.5	13.4	20.3	21.9	18.0	17.7	14.7	23.2	15.7
	Gr (%)	52.4%	49.6%	48.5%	55.8%	47.9%	54.7%	53.5%	57.3%	61.8%	61.6%
7	MLW (mg)	21.2	22.0	21.6	32.9	35.1	30.4	27.8	24.0	37.5	26.1
	Gr (%)	64.3%	63.2%	60.5%	62.5%	60.4%	68.7%	57.0%	62.5%	61.3%	66.4%
8	MLW (mg)	35.2	35.7	34.8	50.8	57.3	47.8	42.5	37.1	55.3	41.0
	Gr (%)	65.9%	62.1%	61.3%	54.2%	62.9%	57.2%	53.1%	54.9%	47.6%	57.1%
9	MLW (mg)	54.4	55.0	54.8	69.8	81.2	69.2	57.6	56.7	77.0	58.7
	Gr (%)	54.7%	54.3%	57.5%	37.6%	41.8%	44.9%	35.5%	52.9%	39.2%	43.3%
10	MLW (mg)	75.0	80.0	80.0	95.0	104.0	95.0	78.0	80.0	101.0	89.0
	Gr (%)	37.9%	45.4%	46.0%	36.1%	28.1%	37.3%	35.4%	41.0%	31.1%	51.6%
11	MLW (mg)	101.2	110.5	105.7	128.8	125.7	119.5	99.9	108.9	130.3	125.7
	Gr (%)	34.9%	38.1%	32.1%	35.6%	20.9%	25.7%	28.1%	36.1%	29.0%	41.2%
12	MLW (mg)	129.4	132.6	133.0	139.4	150.0	141.3	110.1	132.5	137.6	143.7
	Gr (%)	27.9%	20.0%	25.9%	8.2%	19.3%	18.3%	10.2%	21.7%	5.6%	14.3%
13	MLW (mg)	144.7	147.6	147.1	154.5	155.5	156.5	124.1	153.2	155.0	157.2

	Gr (%)	11.8%	11.3%	10.6%	10.8%	3.6%	10.8%	12.7%	15.7%	12.7%	9.4%
14	MLW (mg)	148.7	146.7	156.7	159.5	165.8	165.3	133.5	153.7	156.5	162.4
	Gr (%)	2.8%	-0.6%	6.5%	3.2%	6.7%	5.6%	7.6%	0.3%	1.0%	3.3%
15	MLW (mg)	159.2	164.2	166.6	176.9	161.9	170.2	140.5	150.5	167.0	170.2
	Gr (%)	7.1%	11.9%	6.3%	10.9%	-2.3%	3.0%	5.2%	-2.1%	6.7%	4.8%
16	MLW (mg)	159.2	160.3	171.8	172.0	163.1	172.2	139.1	152.6	153.6	174.5
	Gr (%)	0.0%	-2.4%	3.2%	-2.8%	0.7%	1.1%	-1.0%	1.4%	-8.0%	2.5%
	WMGr (%)								**42.6%**		**45.9%**
17	MLW (mg)	159.3	160.2	177.5	181.7	165.3	163.7	142.4		178.2	
	Gr (%)	0.1%	0.0%	3.3%	5.7%	1.4%	-4.9%	2.3%		16.0%	
	WMGr (%)						**41.7%**				
18	MLW (mg)	162.2	153.2	163.6	191.3	163.1		147.7		168.5	
	Gr (%)	1.8%	-4.3%	-7.8%	5.3%	-1.4%		3.8%		-5.5%	
	WMGr (%)				**44.1%**						
19	MLW (mg)	164.8	163.8	167.4		163.2		142.7		175.4	
	Gr (%)	1.6%	6.9%	2.3%		0.1%		-3.4%		4.1%	
	WMGr (%)							**37.2%**			
20	MLW (mg)	163.4	155.6	162.3		161.0				175.7	
	Gr (%)	-0.9%	-5.0%	-3.1%		-1.3%				0.2%	
	WMGr (%)			**40.9%**		**39.5%**				**39.8%**	
21	MLW (mg)	170.0	168.9								
	Gr (%)	4.0%	8.6%								
	WMGr (%)										
22	MLW (mg)	163.8	160.4								
	Gr (%)	-3.7%	-5.0%								
	WMGr (%)		**45.0%**								
23	MLW (mg)	157.5									
	Gr (%)	-3.8%									
24	MLW (mg)	156.7									
	Gr (%)	-0.5%									
25	MLW (mg)	153.0									
	Gr (%)	-2.4%									
26	MLW (mg)	140.2									
	Gr (%)	-8.4%									
27	MLW (mg)	136.7									
	Gr (%)	-2.5%									
28	MLW (mg)	134.9									
	Gr (%)	-1.3%									
29	MLW (mg)	133.3									
	Gr (%)	-1.2%									
30	MLW (mg)	128.2									
	Gr (%)	-3.8%									
31	MLW (mg)	120.1									
	Gr (%)	-6.3%									
32	MLW (mg)	119.4									
	Gr (%)	-0.6%									
33	MLW (mg)	113.5									
	Gr (%)	-4.9%									
34	MLW (mg)	108.7									
	Gr (%)	-4.2%									
	WMGr (%)	**33.9%**									

Appendix 3

Incubator details, data logger results (Table A21) and disturbances throughout the experiment with relocation of experimental boxes in other incubators (Table A22).

Table A21

Incubator details with Temperature T in °C and Photoperiod. Model Linder fridges are former fridges which were converted to incubators with different fridge models and ages. Light via luminescent light tubes which were mounted on the side throughout the height of the incubator or on the top (ceiling). Leak potential describes the door openings: no, no visible slit, small, visible slits, big, visible opening. Data loggers testo® 174 T were used to record temperature T in °C (± SD) and relative humidity rH in % (± SD) in two periods. Data logging period 1: 16.09.2018 – 08.12.2018. On 01.10.2018 (Incubators 4-9) and 20.11.2018 (Incubators 1-3) water boxes were put in incubators to adjust relative humidity. At these points mealworm developmental stages were similar for Incubators 4-9 and 1-3. Data logging period 2: 09.02.2019 – 04.05.2019, which was not used to calculate the influence of relative humidity (chapter 4.4.) as at this point most mealworms already pupated.

Incubator	T (°C)	Photoperiod	Incubator model	Light	Leak potential	T (°C) ± SD		rH (%) ± SD	
						Data logging period 1	Data logging period 2	Data logging period 1	Data logging period 2
I1	20	16L/8D	Linder fridge	side	no	19.5 ± 0.1	20.7 ± 0.2	51.3 ± 8.1	57 ± 4.9
I2	20	8L/16D	Linder fridge	top	no	20.4 ± 0.3	20.7 ± 0.2	50.6 ± 9.9	no data
I3	20	24D	Linder fridge	side	small	19.1 ± 0.1	19.6 ± 0.2	50.7 ± 8.3	63.1 ± 5.4
I4	25	16L/8D	Linder fridge	side	small	24.8 ± 0.3	23.8 ± 1.6	48.6 ± 8.5	67.1 ± 4.7
I5	25	8L/16D	Linder fridge	side	big	25 ± 0.3	25.1 ± 0.1	44 ± 6.8	49.7 ± 4.9
I6	25	24D	Linder fridge	side	small	25.4 ± 0.3	25.3 ± 0.3	50.8 ± 10.1	57.7 ± 4.2
I7	30	16L/8D	Linder fridge	top	small	30.7 ± 0.7	30.6 ± 0.6	49 ± 12.6	57.4 ± 6.2
I8	30	8L/16D	Linder fridge	top	small	28.1 ± 1.9	no data	51 ± 7	no data
I9	30	24D	Linder fridge	top	small	29.9 ± 0.3	30 ± 0.1	48.7 ± 12	60.9 ± 5.8

Table A22
Incubator details with Temperature T in °C and Photoperiod. Model Linder fridges are former fridges which were converted to incubators with different fridge models and ages. Disturbences and relocation of experimental boxes throughout data collection.

Incubator	T (°C)	Photoperiod	Incubator model	Disturbence	Relocation of boxes
I1	20	16L/8D	Linder fridge	06.02.2019: 35 °C for max 18 hours (no larva died)	06.02.2019: Memmert ICP 500[a]
I2	20	8L/16D	Linder fridge		
I3	20	24D	Linder fridge		
I4	25	16L/8D	Linder fridge	20.11.2018: photoperiod switch stopped, time of constant darkness unknown	13.12.2018: Memmert ICP 500[a]
I5	25	8L/16D	Linder fridge		
I6	25	24D	Linder fridge		
I7	30	16L/8D	Linder fridge		
I8	30	8L/16D	Linder fridge	17.09.2018: no light and 26 °C for approximately four days	17.09.2018: Memmert ICP 500[a]
				26.09.2018: 24 °C for one day	03.10.2018: similar Linder fridge
I9	30	24D	Linder fridge	27.10.2018: 26 °C for one day	27.10.2018: similar Linder fridge

[a]Memmert ICP 500 incubator with no leaking potential and luminescent lights on the side.

Appendix 4

Table of experimental boxes with one or more pair of parents (Table A23). There could have been mealworms with different fathers in every experimental box, if the mother mated before she was randomly selected with another male beetle and put in one experimental box. Statistical test (Welch-test or Wilcoxon-test, alpha = 0.05) of a possible effect of different genotypes (Table A24) needs to be treated with caution as the sample size is low.

Tabel A23

Number of parents per box. If beetles died during the egg laying period they were removed. New beetles and therefore parents were randomly selected from the stock culture and put in this box to ensure enough experimental mealworms per box (New parents). If, at the beginning of data collection, there were less than 20 mealworms in instar four to six in one box, then the box was filled up with mealworms from different boxes within the same incubator (Filled up). Total number of parents depicts the number of parents per box.

Incubator1: 20°C, 16L/8D										
Box (n)	1	2	3	4	5	6	7	8	9	10
New parents										
Filled up	1	2	1			1				
Total number of parents	2	3	2	1	1	2	1	1	1	1
Incubator 2: 20°C, 8L/16D										
Box (n)	11	12	13	14	15	16	17	18	19	20
New parents										
Filled up				1						1
Total number of parents	1	1	1	2	1	1	2	1	1	2
Incubator 3: 20 °C, 24D										
Box (n)	21	22	23	24	25	26	27	28	29	30
New parents										
Filled up	1							1		
Total number of parents	2	1	1	1	1	1	1	2	1	1
Incubator 4: 25°C, 16L/8D										
Box (n)	31	32	33	34	35	36	37	38	39	40
New parents										
Filled up	1									
Total number of parents	2	1	1	1	1	2	1	1	1	1
Incubator 5: 25°C, 8L/16D										
Box (n)	41	42	43	44	45	46	47	48	49	50
New parents								1		
Filled up		1								
Total number of parents	1	2	1	1	1	1	1	2	1	1
Incubator 6: 25°C, 24D										
Box (n)	51	52	53	54	55	56	57	58	59	60
New parents										
Filled up										
Total number of parents	1	1	1	1	1	1	1	1	1	1
Incubator 7: 30°C, 16L/8D										

Box (n)	61	62	63	64	65	66	67	68	69	70
New parents										
Filled up	1								1	
Total number of parents	2	1	2	1	2	1	1	1	2	1
Incubator 8: 30°C, 8L/16D										
Box (n)	71	72	73	74	75	76	77	78	79	80
New parents										
Filled up										
Total number of parents	1	1	1	1	1	1	1	1	1	1
Incubator 9: 30°C, 24D										
Box (n)	81	82	83	84	85	86	87	88	89	90
New parents	1	2	1	2	1	2	2	1	2	1
Filled up										
Total number of parents	2	3	2	3	2	3	3	2	3	2

Table A24

Statistical test for a possible genotype influence. Significant difference between boxes with one pair of parents and boxes with more than one pair of parents (Yes).

Welch-test or Wilcoxon-test, alpha = 0.05.

Incubator	I1	I2	I3	I4	I5	I7
Survival rate	No	No	No	No	**Yes**	No
p	0.067	0.383	0.711	0.711	0.044	1
Developmental time	No	No	No	No	No	No
p	0.635	0.579	0.895	0.541	0.718	0.291
Growth rate	**Yes**	No	No	No	No	No
p	0.013	0.104	0.844	0.516	0.868	0.502