Improving feeding efficiencies of black soldier fly larvae, *Hermetia illucens* (L., 1758) (Diptera: Stratiomyidae: Hermetiinae) through manipulation of feeding conditions for industrial mass rearing

by
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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third-party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

The human population is rapidly expanding and raises several concerns in terms of food security and waste management. To feed the human population, we need to start expanding our horizons in terms of what we eat. Insects may be the answer to this. But due to our many other problems, it helps to create multiple solutions from single ideas that promote green industry and help 'heal' our planet rather than only taking from it. This is where the black soldier fly, *Hermetia illucens* (L., 1758) (Diptera: Stratiomyidae: Hermetiinae) may offer such a solution.

Hermetia illucens is a non-pestilent fly that has spread worldwide due to its many innovative uses, for example used as a type of biological control agent for some filth fly species, recycle 1.3 billion tonnes of annual organic waste globally, create high-grade biodiesel and replace (or at least alleviate the demand) for fish or legume-based animal feeds and lipids. However, very little is known about how the protein and lipid rich larvae can be mass reared for industrial purposes.

This thesis answers questions about the feeding environment and density of *H. illucens* larvae in an industrial setting (i.e. food waste, and larger population sizes). By investigating how feed depth and particle size, feed provisioning rations (i.e. larval densities), and population sizes effect the ability of larvae to develop, survive and feed.

The results of this thesis were finding the optimal feed depths (i.e. 5-10 cm), provisioning rations (125 mg/larva/day) and population sizes (5 000-50 000 larvae per container) of *H. illucens larvae when fed kitchen wastes*. Additionally, two new measures of feeding efficiencies were described (i.e. provisioning ration change and optimal bioconversion deficit) and values for larval aggregation heat were also recorded for the first time. Future recommendations and research that came up during this study is also given to continue furthering an industry capitalising on US$ 750 million lost annually in all waste streams worldwide.

OPSOMMING

Die menslike bevolking is besig om vinnig uit te brei, wat verskeie bekommernisse wek ten opsigte van voedselsekuriteit en afvalbestuur. Om die menslike bevolking te voed, moet ons begin om ons horisonne te verbreed in terme van wat ons eet. Insekte sal waarskynlik die antwoord hierop wees. Maar as gevolg van ons baie ander probleme, help dit om verskeie oplossings te skep vanuit enkele idees wat groen bedryf bevorder en wat help om ons planeet te genees eerder as om daarvan te neem. Dit is waar die swart soldaatvlieg, *Hermetia illucens* (L., 1758) (Diptera: Stratiomyidae: Hermetiinae) 'n oplossing kan bied.

Hermetia illucens is 'n nie-pes vlieg wat wêreldwyd versprei het as gevolg van die vele innoverende doeleindes waarvoor dit gebruik kan word, byvoorbeeld as ’n biologiese beheeragent vir verskeie vullis vliegspesies, vir die herwinning van ’n jaarlikse 1,3 miljard ton organiese afval wêreldwyd, vir die vervaardiging van hoë-graad biodiesel en die vervanging van (of ten minste verligting van die aanvraag na) vis of peulplant gebaseerde veevoere en lipiede. Baie min is egter bekend oor hoe die proteïen- en lipiedryke larwes in groot maat geteel kan word vir industriële doeleindes.

Hierdie tesis beantwoord vrae oor die voedingsomgewing en bevolkingsdigtheid van *H. illucens* larwes in 'n industriële omgewing (m.a.w. voedselafval en groter bevolkingsgroottes). Dit is gedoen deur te ondersoek hoe voerdiepte en partikelgrootte, voer voorsieningsrantsoene (d.w.s. larwedigthede), en bevolkingsgroottes die vermoë van larwes om te ontwikkel, oorleef en voed, beinvloed.

Die resultate van hierdie tesis het die optimale voerdieptes (d.w.s. 5-10 cm), voorsieningsrantsoene (125 mg / larwe / dag) en bevolkingsgroottes (5 000-50 000 larwes per houer) van *H. Illucens* vasgestel vir larwes wat met kombuisafval gevoer is. Daarbenewens is twee nuwe maatreëls van voedingsdoeltreffendheid beskryf (d.w.s. voorsieningsrantsoen verandering en optimale bio-omsetting), en waardes vir larwale samedrommingshitte is ook aangeteken vir die eerste keer. Toekomstige aanbevelings en navorsing wat in hierdie studie na vore gekom word ook gegee om voort te gaan om 'n

bedryf te bevorder wat kapitaliseer op 750 miljoen USD wat jaarliks in afvalstrome wêreldwyd verlore gaan.

DEDICATION

For Jeannette Brits and my family.

Thank you for always making me grow and

keeping my eyes on God

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To my mother, Jeannette Brits, there are no words to express what you have given me in this life so I will simply say thank you with all my heart. I would not be the man or have had any of the opportunities I have today if you did not believe in me and help me every step of the way. Thank you for all the slaps on the ear when I needed them and the love you have shown me. Thank you for being my example of hard work and dedication and for being my hero when I needed it most. To Andrea, Leon and Kaid Swanepoel thank you for being my support and rock when I struggled and felt like giving up. I would not be writing this if it weren't for you guys. I love you all. To my father, Mark Brits, and grandfather, Oswald Barker, I hope you are looking down proud, thank you for shaping my character for all the years I got to spend with you both. You are both missed, rest in peace. Finally, to my Lord and saviour Jesus Christ, I give all the glory and honour to you. Thank you for your grace and mercy and may I use all my blessings for your cause in the future to come.

LIST OF PUBLICATIONS

BRITS, D, PRYKE, J S, RICHARDS, C S & VILLET, M H. The effect of food availability on the development and feeding efficiencies of black soldier fly larvae populations, *Hermetia illucens* (L., 1758) (Diptera: Stratiomyidae). Submitting to the Journal for Insects as Food and Feed.

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LIST OF ABBREVIATIONS

$	American Dollars
AAIS	Academy for Advanced Interdisciplinary Studies
AD	Approximate Digestibility
AFMA	Animal Feed Manufacturers Association
ANOVA	Analysis of Variance
B	Breadth
ca.	Approximately
cm	Centimeters
CO_2	Carbon dioxide
Conf.	Conference
D.Phil.	Doctor of Philosophy
D.Sc.	Doctorae Philosophae
DM	Dry matter
ECD	Efficiency of Conversion of Digested-feed
ECI	Efficiency of Conversion of Ingested-feed
EFSA	European Food Safety Authority
EIIC	Electronic International Interdisciplinary Conference
et al.	*et alli*
FAO	Food and Agriculture Organisation
Faun. Abh.	Faunistica Abhandlungen
g	Grams
GLM	General Linear Models
h	Hours

H	Height
H. illucens	*Hermetia illucens*
Lt	Litres
L	Length
L.	Linneaus
L5	Fifth Instar Larva
M. domestica	*Musca domestica*
M.Sc.	Master of Science
MANOVA	Multivariate Analysis of Variance
Max.	Maximum
mg	Milligrams
no.	Number
NRF	National Research Foundation
OBD	Optimal Bioconversion Deficit
pH	Potential of Hydrogen
Ph.D.	Philosophae Doctorae
PLoS ONE	Public Library of Science One
PRC	Provisioning Ration Change
Proc.	Proceedings
r	Radius
Revista de la S.E.A.	Revista de la Sociedad Entomológica Argentina
RH	Relative Humidity
RMANOVA	Repeated Measures Analysis of Variance
SA	South Africa
SA: Vol.	Surface Area: Volume
SD	Standard Deviation

SE	Standard Error
sp.	Species (singular)
spp.	Species (plural)
UK	United Kingdom
UN	United Nations
USA	United States of America
WM	Wet Matter
WR	Waste Reduction
Z. ang. Ent.	Zeitschrift fuer Angewandte Entomologie

CHAPTER 1:

INTRODUCTION AND LITERATURE REVIEW

1. Introduction

Black soldier fly, *Hermetia illucens* (L., 1758) (Diptera: Stratiomyidae: Hermetiinae) research to date: prospects for the future

The human population is growing at a rapid rate with the global population at more than 7.3 billon people and expected to reach 9.7 billion by 2050 (UN 2015). The population increase will inevitably put further stress on global food security (FAO 2002). Two primary concerns arise within the consumer chain mainly regarding protein supply (particularly fish production) (Merino et al. 2012; Thurstan & Roberts 2014; FAO 2016) and food and agricultural wastage (Qotole et al. 2001; FAO 2011; Nahman 2011; Nahman et al. 2012; The Economist 2014; Alooh 2015; Davison 2015; Salomone et al. 2016). These two areas are believed to be the key to alleviating the pressure that our increasing population size will experience in the next 33 years (FAO 2011, 2013, van Huis et al. 2013, 2015).

1.1 Fish exploitation and fishmeal

Recently there has been a significant increase in the demand for protein and lipid sources throughout most developed and developing countries, particularly from fish (FAO 2002, 2013, 2016). This has led to a rapidly expanding aquaculture industry, which requires wild caught fish as a food source, and is said to exceed the wild fish production by 2025 (Merino et al. 2012; FAO 2016). The increasing price of fishmeal and fish oil (i.e. lipids) required for aquaculture, due to overexploitation, may lead to making fish inaccessible to many (Merino et al. 2012; Thurstan & Roberts 2014; FAO 2016). Additionally, 30% of the 167.2-million-ton fish catch (55.86% wild catch and 44.14% aquaculture) is used to feed other livestock which is a diversion of food that could be used for human consumption (FAO 2002, 2004, 2016).

The use of alternative feedstocks for aquaculture has been reviewed in depth for plant and many insect-based alternatives, which in turn would allow more fish caught to be used for human consumption

(Bondari & Sheppard 1981, 1987; Alvarez et al. 2007; Devic et al. 2012; Karapanagiotidis et al. 2014). Insect-based protein and lipids have added benefits in that they use less land than crop production (as they can be mass produced in small facilities and plant proteins are often not ideal for fish feeds (Olsen & Hasan 2012)) and they have potential to solve waste management concerns in the process (Bondari & Sheppard 1981; van Huis et al. 2013; Tomberlin et al. 2015).

1.2 Organic waste and its management

Food production processes waste about one-third of all food produced either pre- or post-consumption (FAO 2011; Alooh 2015). This equates to roughly 1.3 billion tonnes of organic waste annually (FAO 2011). Economically, that accounts for US$ 750 million per annum of food lost (The Economist 2014). Some authors have compared that this amount could feed three billion people in the equivalent time frame if it were accessible (FAO 2011; The Economist 2014).

Not only does this have social and economic impacts but organic landfill waste accounts for ca. 19% of all anthropogenic greenhouse gas emissions of methane and carbon dioxide worldwide thus contributing to global warming on a large scale (Hilger & Humer 2003; Nahman 2011; Nahman et al. 2012; Alooh 2015). In fact, one ton of organic waste produces as much as 4.5 tonnes of CO_2 (FAO 2011; Alooh 2015). Additionally, food wastage has disamenity costs for waste management (Nahman 2011) and causes inefficiencies and losses in other precious commodities such as water and oils (Nahman et al. 2012). For example, the US alone uses 300 million barrels of oil to produce wasted food and that wasted food occupies more than one-third of all agricultural land using 250 km^3 of water (Alooh 2015). These are heavy costs to pay but the case study of Cape Town (SA) by Nahman (2011) shows that it is possible to significantly cut back on many of these impacts by incorporating energy recovery bodies into current landfills and waste management practices. While this is useful economically, it does not solve the issue of waste and lost nutritive and economic value. This is where vermiculture may be beneficial.

1.3 Vermiculture

Vermiculture is the use of Oligochaetes (e.g., earthworms) or insects (most commonly flies (Diptera)) to compost and bioconvert organic wastes into useful products such as fertilisers and protein from the larval muscle tissue that they generate through growth. However, many species of flies and earthworms cannot process a wide range of organic wastes and need specific nutrition to be effective bioconverters (Morgan & Eby 1975; Latsamy & Preston 2008; Morales & Wolff 2010; Yadav et al. 2010; Kassam 2012; Zhang et al. 2012; Yang et al. 2012). In addition, some are human pests and disease vectors (Mitchell et al. 1974; Singh & Fotedar 1992; Sasaki et al. 2000; De Jesús et al. 2004). Much research has recently been focussed on the black soldier fly, *Hermetia illucens* (L., 1758) (Diptera: Stratiomyidae: Hermetiinae) that over 100 years ago, was said to have no economic value (Dunn 1916; Bradley 1930).

The rest of this review will look at the history and many uses of this fly species and identify research areas and methodology that need addressing.

1.4 Taxonomy and biogeography

The black soldier fly, *Hermetia illucens* (L, 1758) (Diptera: Stratiomyidae: Hermetiinae) originates from Central America (Dunn 1916; Johannsen 1922; Copello 1926; James 1935, 1960, 1981; McFadden 1967; Leclercq 1969, 1997; Benelli et al. 2014). It has undergone many taxonomic name changes since it was originally described by Carl Linnaeus in 1758 but was reclassified from Muscidae (which most flies were classified as during this era) to Stratiomyidae in 1805 by Johan Fabricius, and the now accepted name developed after Andrés Copello's name change incorporated Linnaeus' *illucens* name once again (Oliveira et al. 2015).

Hermetia illucens spread rapidly throughout the world during and after World War II through incidental introductions, causing them to occur throughout the world, particularly throughout Europe, Asia and Australia (May 1961; Leclercq 1969; Lien & Chien 1974; Hauser & Rozkošný 1999; Üstüner et al. 2003; Bal et al. 2007; Heo et al. 2007; Chin et al. 2008; Nartshuk 2009; de Groot & Veenvliet 2011; Ndueze et

al. 2013; Roháček & Hora 2013; Irish et al. 2013; Benelli et al. 2014). This has resulted in the fly naturalising in most geographic locations within the warm temperate and tropical regions (Leclercq 1969; Benelli et al. 2014; Marshall et al. 2016). A few reviews (Copello 1926; Leclercq 1969; Marshall et al. 2016) and taxonomic research (Malloch 1917; Johannsen 1922; James 1935, 1960, 1981; Bohart & Gressitt 1951; McFadden 1967; Rozkošný 1982; Hauser & Rozkošný 1999; Woodley 2000, 2009; Kehlmaier 2004; Velasquez et al. 2010; Mason 2013) quantified their distribution worldwide to date and detail some interesting cases of their occurrence particularly in European forensic accounts (Benelli et al. 2014).

1.5 Non-pest status

Hermetia illucens is considered a non-pestilent fly as the adults do not feed (Sheppard et al. 2002) and are not often found in human settlements, except in exposed pit latrines (Bradley 1930; Lien & Chien 1974; Irish et al. 2013) or on animal farms (Brady & LaBrecque 1966; Peck & Anderson 1970; Axtell 1986; Newton et al. 2005; Yu et al. 2011) where they utilise manure as food. Since the larvae rarely encounter humans (Meleney & Harwood 1934; Werner 1956; Lee et al. 1995; Yoneda et al. 1998; Calderon-Arguedas et al. 2005), the species is not considered a pest or disease vector as this limits their possibility of disease transmission or nuisance habits compared to synanthropic Diptera such as housefly, *Musca domestica* (James 1947). Additionally, *H. illucens* larvae has been shown to produce antimicrobial substances that fight disease (Erickson et al. 2004; Liu et al. 2008; Choi et al. 2012; Gabler 2014; Leong et al. 2015b) and are implicated in the control of other flies such as *M. domestica* (Furman et al. 1959; Kilpatrick & Schoof 1959; Sheppard 1983; Bradley & Sheppard 1984), which further emphasizes their non-pest status.

1.6 Feeding habits and environmental conditions

Hermetia illucens larvae are voracious feeders of a wide range of organic materials including manure, decaying plant materials, and carrion (Newton et al. 2005; Kalová & Borkovcová 2013; Žáková &

Borkovcová 2013; Paz et al. 2015). Not only do they feed on these materials but they do it very efficiently, being recorded to reduce their feed by up to 60% (Newton et al. 2004; Myers et al. 2008; Diener et al. 2011; Zhou et al. 2013; Banks 2014; Oonincx et al. 2015b; Tschirner & Simon 2015; Salomone et al. 2016), and bioconvert more than 30% of that into their own body mass under optimal conditions (Zheng et al. 2012a; Banks et al. 2014; Li et al. 2015; Holmes et al. 2016).

The larvae have been shown to withstand temperatures ranging from 16 - 40°C (Harnden & Tomberlin 2016; Holmes et al. 2016) with an optimal for development and size at 27.5°C (Tomberlin et al. 2009). A single *H. illucens* will take a maximum of 55 days to complete its lifecycle (Tomberlin et al. 2009). In addition to temperature, a relative humidity between 60-90% is common practice in *H. illucens* literature (Sheppard et al. 2002; Myers et al. 2008; Pujol-Luz et al. 2008; Diener et al. 2009; Erens et al. 2012; Roháček & Hora 2013; Stankus 2013; Zhou et al. 2013; Nguyen et al. 2013; Makkar et al. 2014; Banks et al. 2014; Oonincx et al. 2015a; Holmes et al. 2016; Nakamura et al. 2016) and has been shown to have significant effects on the pupal and adult stages (Holmes et al. 2012). Additionally, adults require bacteria to attract females for oviposition (Zheng et al. 2013) while males need lekking sites to establish successful mating (Tomberlin & Sheppard 2001) and correct lighting conditions (Zhang et al. 2010) to reproduce and oviposit efficiently.

The duration from egg hatch to prepupae is ca. 21 days (Tomberlin et al. 2009). Larvae require a similar feed moisture content to that of their humidity but can survive anywhere between 40-80% moisture. Finally, the larvae nutritionally consist of 44.1±9.1% protein and 27.6±9.6% lipids (sourced from the following: Bondari & Sheppard 1987; Ravindran & Blair 1993; Barry 2004; Newton et al. 2004; St-Hilaire et al. 2007; Diener et al. 2009; Muin et al. 2012; Park et al. 2013; Finke 2013; van der Spiegel et al. 2013; Rumpold & Schlüter 2014; Bosch et al. 2014; Karapanagiotidis et al. 2014; Mlcek et al. 2014; Barroso et al. 2014; Sánchez-Muros et al. 2014; Oonincx et al. 2015a; Shakil Rana et al. 2015; Stamer 2015; Veldkamp & Bosch 2015; Tschirner & Simon 2015; Surendra et al. 2016) and tend to feed in

mediums with similar macronutrient balances (St-Hilaire et al. 2005; Diener et al. 2009; Park et al. 2013a; Kenis et al. 2014; Tschirner & Simon 2015; Maquart et al. 2016; Surendra et al. 2016).

1.7 *Hermetia illucens* in the scientific literature

Hermetia illucens larvae produce many by-products during their development and have been researched at increasing levels throughout the past 100 years. Literature was searched using the terms, 'black soldier fly' and '*Hermetia illucens*' on Google Scholar, Research Gate, and Stellenbosch University's worldwide online library catalogue. Two-hundred-and-thirty-two relevant papers were sourced and categorised per the overall theme of the papers, whether they were direct or indirect mentions, reviews, feeding trials, product development, life history (i.e. anything to do with physiology, environmental interactions or behaviour of *H. illucens)* or mass rearing based papers. Figure 1.1: The number of research papers involving *Hermetia illucens* in the past 100 years (1916-2016) shows the variation in literature over the past 100 years and the general categories of literature. While biogeographic and taxonomical research of *H. illucens* has been evenly spread throughout time, the introduction of other topics such as by-product identification and creation, life history and mass rearing only occurred after 1960. Literature also significantly increased at the turn of the 21st century, producing more than 76% of the literature (Figure 1.1). Of these, life history, mass rearing, insect feed reviews mentioning *H. illucens*, products and waste management using *H. illucens* rank in the top five topics during the last two decades in the order of significance given.

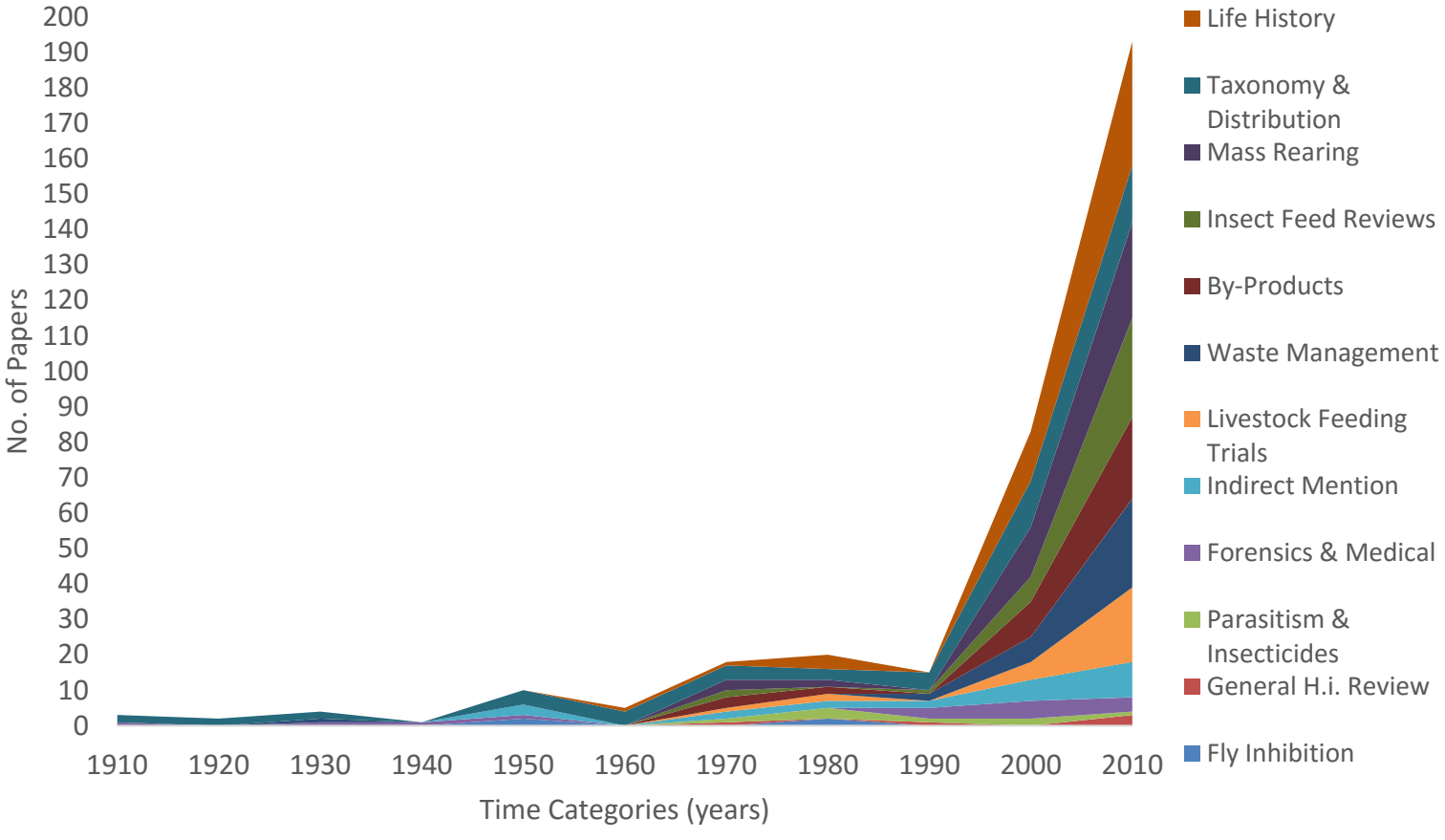

Figure 1.1: The number of research papers involving *Hermetia illucens* in the past 100 years (1916-2016) classified into twelve categories.

1.8 Uses of *Hermetia illucens* through history

1.8.1 Forensics and housefly control

Hermetia illucens has been found on many cadavers through much of the Central Americas (Dunn 1916; Calderon-Arguedas et al. 2005; Pujol-Luz et al. 2008) and has been used in numerous forensic investigations (Lord et al. 1994; Heo et al. 2007; Ahmad & Ahmad 2009; Velasquez et al. 2010; Vanin et al. 2011; Ndueze et al. 2013). The flies preferentially colonise later stages of corpse decomposition (Lord et al. 1994; Heo et al. 2007; Pujol-Luz et al. 2008; Ahmad & Ahmad 2009; Vanin et al. 2011; Ndueze et al. 2013), but Tomberlin et al. (2005) shows that it can occur as early as the first week of decomposition. *Hermetia illucens* has been used for forensic purposes in the United States (Lord et al.

1994), South America (Pujol-Luz et al. 2008), Europe (Velasquez et al. 2010; Vanin et al. 2011), Africa (Ndueze et al. 2013) and South East Asia (Heo et al. 2007; Ahmad & Ahmad 2009).

The inhibition of *Musca domestica* due to the presence of *H. illucens* in poultry houses was first seen by Furman et al. (1959) and confirmed multiple times directly (Kilpatrick & Schoof 1959; Sheppard 1983; Bradley & Sheppard 1984) and indirectly (Axtell & Edwards 1970, 1983; Axtell 1986). Very little is known of the mechanism by which *H. illucens* limits *M. domestica* activity but some authors speculate that it is due to *H. illucens* larvae changing the feed to not suit other flies or possibly due to adults or conspecifics emitting deterring substances (Zheng et al. 2013).

1.8.2 Biodiesel

Hermetia illucens larvae have been shown to be high in lipids that are a good source for high-grade biodiesel when fed organic fraction, dairy manure or organic kitchen wastes (Li et al. 2011a, 2011c, 2015; Zheng et al. 2012b; Leong et al. 2015a). The lipid yield can produce as much as a 96% biodiesel yield when processed (Li et al. 2011a). This is a very new field and seems to be expanding mainly in China and South America, where some recent reviews of biodiesel from insects are mentioned (Li et al. 2011b; Manzano-Agugliaro et al. 2012; Pinzi et al. 2014).

1.8.3 Antimicrobials, enzymes, soaps and detergents

Several enzymes have been identified in *H. illucens* that assist in breaking down the three major macronutrients using amylases, proteases and lipases (Kim et al. 2011a, 2011b; Park et al. 2013b).

Like many flies, *H. illucens* has several antimicrobial properties when feeding in a waste medium or in the presence of specific pathogens (Erickson et al. 2004; Liu et al. 2008; Choi et al. 2012; Gabler 2014; Lalander et al. 2014; Leong et al. 2015b). Their distinct effect on *Salmonella* spp. in chicken manure and other wastes has been studied several times (Erickson et al. 2004; Gabler 2014). Choi et al. (2012) also showed that extracts of *H. illucens* larvae were effective at reducing general gram-negative bacteria.

Leong et al. (2015b) showed that the larvae of *H. illucens* contain high levels of Lauric acid that has antimicrobial and antifungal properties and therefore could be useful for detergent and soap production.

1.8.4 Fertilisers and waste management

Hermetia illucens was first known to inhabit manure and gained a lot of interest around this indirectly due to fly control studies (Schoof & Siverly 1954; Kilpatrick & Bogue 1956; Kilpatrick & Schoof 1957; Lien & Chien 1974), but also because of their ability to reduce the amount of waste present thus acting as manure management agents in confined animal feeding operations (Bradley 1930; Booram et al. 1977; Sheppard et al. 1994; Sheppard 2002; Newton et al. 2004, 2005, Banks 2012, 2014, Lalander et al. 2013, 2015; Diener et al. 2014). This has been reviewed and studied since the 1970s (Booram et al. 1977) and has since expanded to the management of organic wastes such as kitchen wastes (Barry 2004; Diener et al. 2011; Nguyen et al. 2015; Salomone et al. 2016), palm kernel wastes (Hem et al. 2008; Arief et al. 2012), biodiesel fraction (Zheng et al. 2012b) and vegetable wastes (Paz et al. 2015) to name a few.

The larvae are generally able to efficiently reduce waste by as much as 60% (Newton et al. 2004) and the leftover residue is a suitable and safer fertiliser for use in plant growth (Choi et al. 2009; Green & Popa 2012), especially when exuviae of the larvae are added to the fertiliser (Lee et al. 2013). Additionally, this fertiliser can reduce the number of organic leachates produced from organic wastes, which when not treated with *H. illucens*, cause major environmental impacts for surrounding and immediate ecosystems (Green & Popa 2012).

1.8.5 Feed and food

Hermetia illucens has been repeatedly shown to be a rich source of protein and lipids with a high amino and fatty acid complex that can be enhanced and manipulated under the correct conditions to exceed fishmeal and other feeds in some aspects of their composition, having some of the best compositions among insects for food and feed, making the species of significant interest (Ravindran & Blair 1993; Erens et al. 2012; Makkar 2012; van Huis et al. 2013, 2015; Rumpold & Schlüter 2013; Finke 2013; van

der Spiegel et al. 2013; Vantomme et al. 2014; Kenis et al. 2014; Mlcek et al. 2014; Riddick 2014; Barroso et al. 2014; Sánchez-Muros et al. 2014; Anankware et al. 2015; Stamer 2015a; van Huis 2015; European Food Safety Authority 2015). The larvae, prepupae and pupae of *H. illucens* have been successfully fed to fish (Bondari & Sheppard 1981, 1987; St-Hilaire et al. 2005; Moreau 2009; Sealey et al. 2011; Kroeckel et al. 2012; Park et al. 2013a; Stankus 2013; Karapanagiotidis et al. 2014; Stamer et al. 2014; Barroso et al. 2014; Tran et al. 2015; Webster et al. 2015; Tschirner et al. 2016), poultry and other birds (Fujita & Higuchi 2005; Kvist 2012; Manangkot et al. 2014; Widjastuti et al. 2014; Pieterse et al. 2014; Leiber et al. 2015; Veldkamp & Bosch 2015; Cullere et al. 2016; Maurer et al. 2016; Tschirner et al. 2016), swine (Newton et al. 1977; Kortelainen et al. 2014; Veldkamp & Bosch 2015), pets (Bosch et al. 2014), frogs (Dierenfeld & King 2008 cited in Finke 2013), alligators (Bodri & Cole 2007) and even a biological control mite (Nguyen et al. 2016), seeing no real changes at certain inclusion rates of larvae meal compared to fishmeal for all of the above.

Its' use as fish feed has been of specific interest due to aquaculture concerns, mentioned in *1.1.Fish exploitation and fishmeal*, and meta-analysis of the data on feeding trials using *H. illucens* (Figure 1.2). Most prominently several reviews have studied their safety, nutrition and future using several different scopes (Bondari & Sheppard 1981; St-Hilaire et al. 2005; Sheppard et al. 2007; Park et al. 2013a; Barroso et al. 2014; Tran et al. 2015; Charlton et al. 2015; Tschirner et al. 2016). This is most likely due to the poor perception of insects as either feeds or food that is still persistent in western culture today (Defoliart 1999).

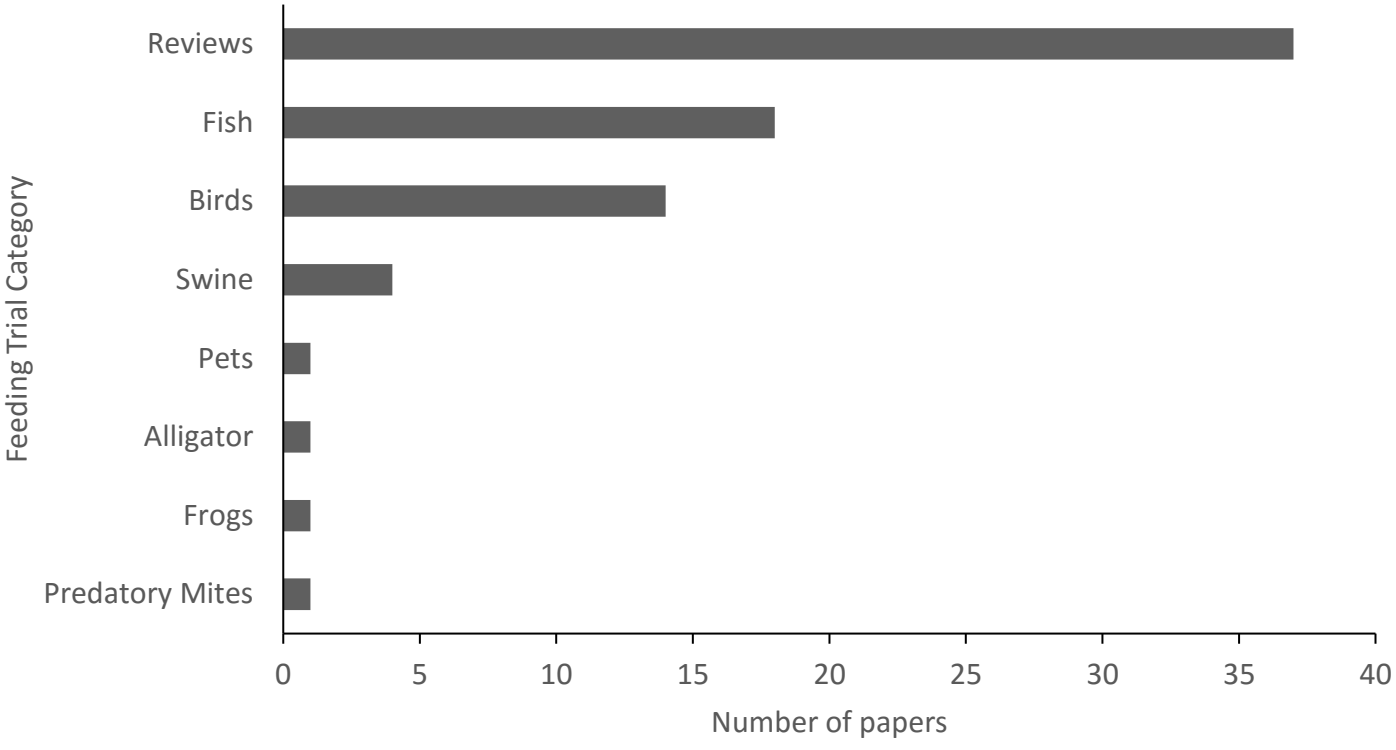

Figure 1.2: Number of *Hermetia* meal feeding trials conducted on specific groups of animals and/or livestock as well as the number of insect feed and food reviews mentioning *H. illucens* in the past 100 years.

1.9 Gaps in literature

1.9.1 Legislation

Not only is public perception a concern in *H. illucens* becoming mainstream in food and feed security, but there are also several other problems. A number of authors have stated that legislation in most major countries restricts insects as use as animal feeds, although many have stated that this will change in coming years and will not hamper the growth of this industry (FAO 2002, 2004; Erens et al. 2012; Rumpold & Schlüter 2013; van der Spiegel et al. 2013; Makkar et al. 2014; Vantomme et al. 2014; Anankware et al. 2015; Stamer 2015a, 2015b; Telfser 2015; Tomberlin et al. 2015; Tran et al. 2015; van Huis 2015; Veldkamp & Bosch 2015; Yen 2015; European Food Safety Authority 2015; Evans et al.

2015; Jozefiak & Engberg 2015; Charlton et al. 2015; van Huis et al. 2015; Drew & Pieterse 2015; Salomone et al. 2016; Cullere et al. 2016).

1.9.2 Increasing the scale of production (up-scaling)

Much of the literature has not looked at scaling up the process of organic waste management to industrial scales. Those that have, although generally only from conference proceedings, stated that there is major instability in larval production under natural conditions (Devic et al. 2014; Maquart et al. 2016) and have shown varying levels and methods of larval feeding efficiencies possibly due to this. One would assume that a firm understanding of life history and how to mass rear *H. illucens* would be necessary to create a stable state population growth (Birch 1948). While research has mainly focussed on life history and mass rearing (Figure 1.3), a small proportion of literature (7.8% overall) has focussed on larval life history even though most literature is larval orientated (Figure 1.4). This is therefore a gap in the literature which needs to be addressed.

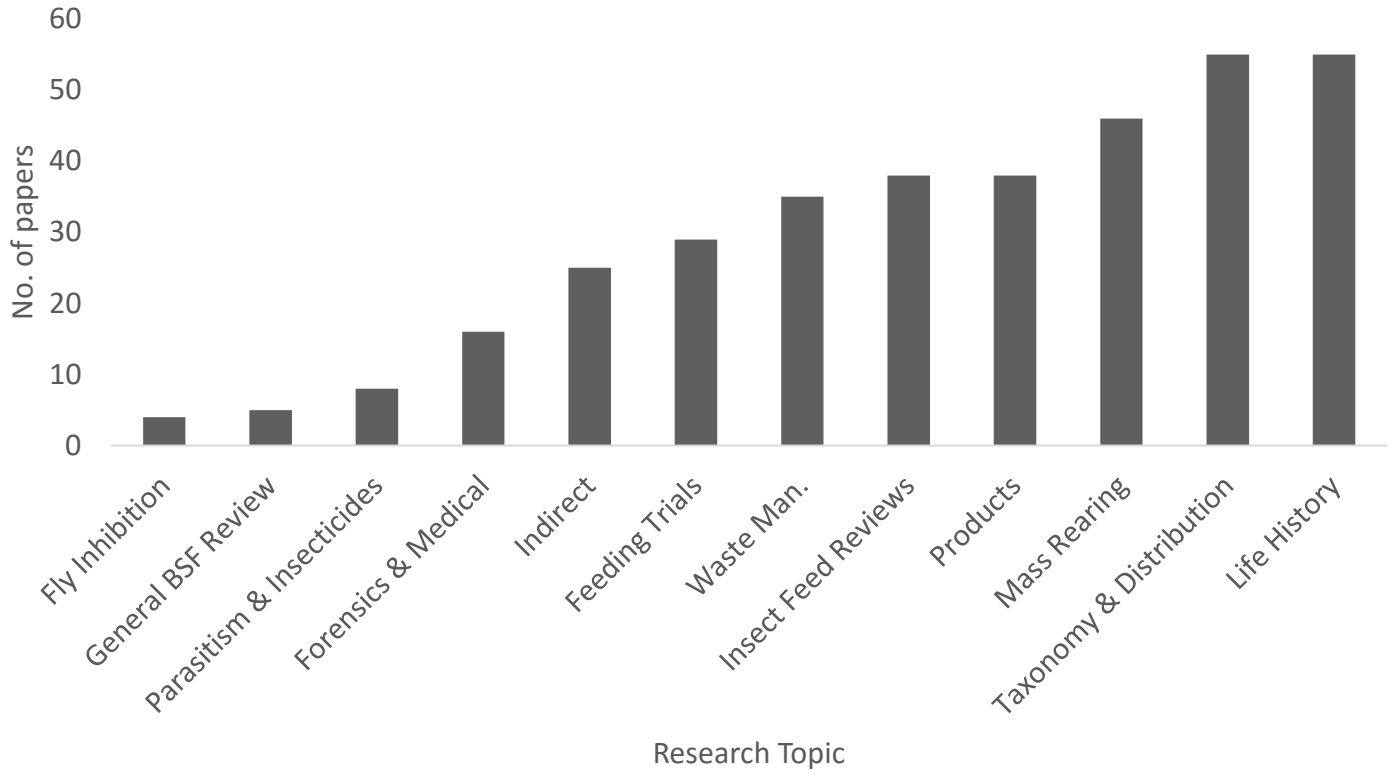

Figure 1.3: Summary of the assigned subdivisions of literature over the past 100 (1916-2016) years of *H. illucens* research.

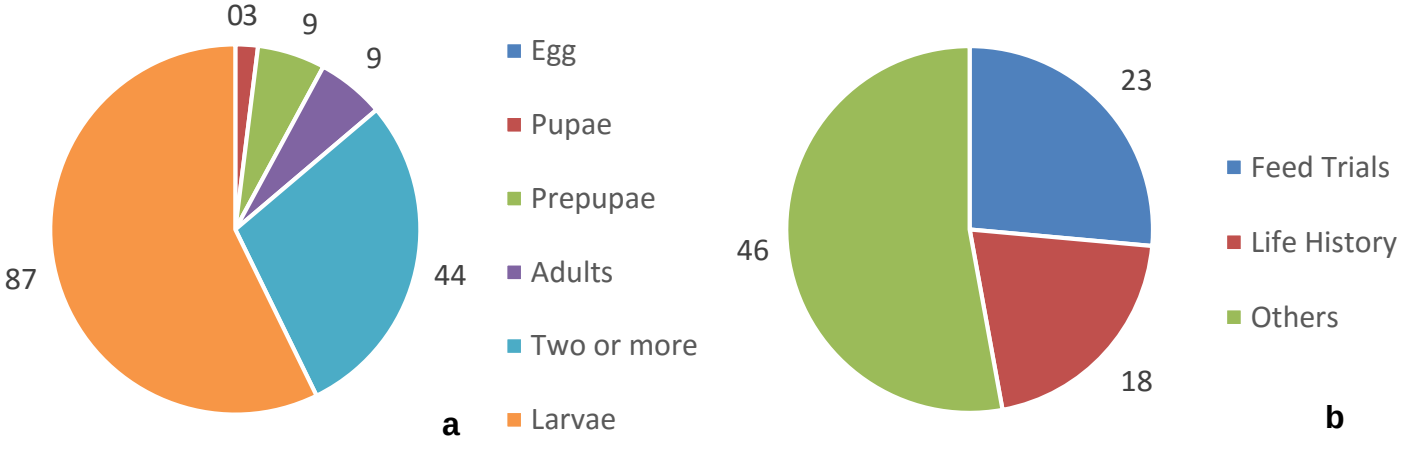

Figure 1.4: a) Proportion of literature assigned to each life stage of *H. illucens* or multiple life stages in the literature on the species to date, b) The proportion of literature with which larval research has focussed on in the past 100 years.

1.9.3 Intraspecific competition

A major gap in literature is the lack of focus on the role of intraspecific competition between larvae. Many studies have shown that insects are significantly affected by intraspecific competition in many forms such as feed restriction and overcrowding (Wasti et al. 1975; Conde & Rabinovich 1979; Poirier & Borden 1992; Barnard & Geden 1993; Applebaum & Heifetz 1999; Stockley & Seal 2001; Dukas et al. 2001; Agnew et al. 2002; Green et al. 2003; Hooper et al. 2003; Guedes et al. 2007; Kivelä & Välimäki 2008; Burrack et al. 2009; Dmitriew & Rowe 2011; Mitchell-Foster et al. 2012). Diener et al. (2009) showed that *H. illucens* larvae are affected by changes in feed provisioning, and feed most efficiently when given 100 mg/larva/day at regular intervals. However, while this is a good indicator of fitness when food is limited, there is no understanding of how this will change when spatial parameters and larval proportions are changed. This would be vital to understand for the future stability in larval production for industrial mass rearing of *H. illucens* as it is most likely to be more variable in the number of larvae that can be produced rather than in the amount of waste that can be sourced. The continual replacement of feed throughout feeding has never been shown to be biologically beneficial, or not, in literature but has somehow developed as a standard protocol for feeding in literature (Sheppard et al. 2002; Diener et al. 2009). Additionally, no literature, to my knowledge, has shown that larvae of *H. illucens* are negatively affected by feeding in one food source for their whole lifecycle.

Not only are the larvae themselves affected by their feeding environment, but the feed too will change over time as the dimensions of the feeding environments change thus changing the interactions between the feed with the larvae (Lardé 1989, 1990; Hooper et al. 2003; Vamosi 2005; Guedes et al. 2007). For example, Lardé (1989) showed that a feed of coffee pulp formed anaerobic zones when feed depths of 20 cm were used in natural conditions and larvae of *H. illucens* could not feed in these zones either due to bacterial activity or inability to access it. This in turn could affect someone looking to combine more food in deeper containers with the optimal feeding rates from Diener et al. (2009) and find odd results due to a flawed assumption made about the access of the feed to the larvae.

1.9.4 Scope of life stage studies

Looking at a single life stage allows for a more dynamic evaluation of specific interactions between the larvae themselves and their feed (examples include Poirier & Borden 1992; Barnard & Geden 1993; Dukas et al. 2001; Green et al. 2003; Hooper et al. 2003; Reim et al. 2006; Mitchell-Foster et al. 2012). This could effectively increase the understanding of nutrient utilisation by *H. illucens*, thus directly affecting production of products such as protein and lipids of larvae. If these competition or density-related issues can be understood, it may be possible to better understand nutritional roles of feed in the larvae reaching their full genetic potential (Elsjé Pieterse, pers. comm.) for food utilisation under mass rearing conditions.

1.10 Applied feeding efficiency

A final concern is the varying methods of measuring feeding efficiencies in *H. illucens*. Calculations used do vary depending on the experimental design or setup of rearing conditions in many cases. However, under applied conditions, certain calculations become redundant due to the time they take to collect for little insight into the life history of the organism. Scriber & Slansky (1981) give a good overview of all possible quantitative methods for evaluating feeding efficiencies specifically in immature insects. Therefore, due to the above-mentioned factors, only certain calculations were chosen that would be beneficial in a time-pressed industrial environment.

Some that are common to literature and industry in animal rearing terms are waste reduction (or approximate digestibility (AD) in Scriber & Slansky (1981)) and bioconversion (or efficiency of conversion of ingested feed (ECI) in Scriber & Slansky (1981)) used in *H. illucens* literature and will be referred to as waste reduction and bioconversion. Literature also rarely discusses how survival and development may affect feeding efficiencies or general life history in *H. illucens* (Fatchurochim et al. 1989; Myers et al. 2008; Nguyen et al. 2013; Akutse et al. 2015; Leong et al. 2015b; Oonincx et al. 2015a; Tschirner & Simon 2015). Diener et al. (2009) also speak of feeding rates, this however requires

caution when being quoted in other literature as this assumes that the amount of food given to the larvae is all being eaten; when in fact, it may only be provisioned to the larvae for a set period and their feeding on the feed will be determined by their current conditions and ability to access the nutrients under those conditions. These conditions are therefore poorly understood and need further research as shown by the extensive literature reviewed above (Figures 1.3 and 1.4).

1.11 Thesis outline

Therefore, while there are considerable uses and applications of many facets of *H. illucens*, there are also a plethora of questions that still need answers and gaps that need filling before a stable state of feeding efficiency (and nutrient utilisation) can be achieved.

Thus, the aim of this thesis will be to better understand how intraspecific competition and feeding environment have vital roles on the larval life history and feeding efficiencies using applied conditions to simulate actual, up-scaled environmental conditions.

Factors that will be investigated will be feed depth and feed particle size and its effect on feed accessibility and abovementioned factors; provisioning rations under set feeding regimes (from Diener et al. 2009 feeding calculations); and finally, population scaling effects on feeding efficiencies and harvesting processes.

Not only will these variables be investigated experimentally but an applied, approach will be taken to make results applicable to an organic, industrial-sized, waste upcycling plants. Thus, organic waste from select sources will be used and conditions will be controlled to a major extent but will be constantly monitored and related back to the data produced from this thesis.

Factors such as waste reduction, bioconversion, survival, development under set time periods and new novel quantitative methods to be explored to evaluate feeding of *H. illucens* larvae. Additionally, since experiments will mimic industrial conditions, there may be variability within the setups and therefore a constant record of ambient conditions (temperature, relative humidity, pH, feed moisture and nutrient

content) will be monitored and compared within and across experiments to evaluate their impact on the factors investigated, if any.

These variables will contribute towards a better understanding of provisioning rations and environmental effects on *H. illucens* larval feeding abilities. This will ultimately culminate in a modular understanding of a better enclosure for larval feeding under industrial conditions and, in part, contribute to a more holistic understanding of larval feeding and thus product raw material production for the growing industry and its' need for stable larval growth.

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CHAPTER 2:

DEPTH AND PARTICLE SIZE

2. The effect of food availability on the development and feeding efficiencies of black soldier fly larvae, *Hermetia illucens* (L., 1758) (Diptera: Stratiomyidae)

2.1 Abstract

Industrial mass rearing of *H. illucens* has recently become economically important and so much research is needed as to how to mass rear them at these scales. One such factor is how the depth and particle size of the larval feed effects their feeding ability and access to food. This study investigated how these two variables affect waste reduction, bioconversion, feed depth access, final larval mass, survival and development. Two new calculations for feeding efficiencies were also introduced: provisioning ration change and optimal bioconversion deficit. Larvae were reared under controlled conditions over two trial periods separated by a week. Treatments were 5-20 cm feed depths at 5 cm increments and three different particle size treatments: control, sieved and blended feeds. Larva-free controls were also included to account for bacterial and environmental breakdown of the feed. Larvae were measured for feed depth and aggregation/feed temperature the day after being placed on the feed and measured every two days until the 15th day of experimentation. Larvae were then removed using a wet separation technique and weighed and all feeding efficiencies and variables were calculated. Results showed that all variables had significant effects for both feed depths and particle sizes. Larvae performed best below 10 cm in terms of survival feed access and feeding efficiencies but developed significantly faster below 5 cm depths. These results show the importance of these two variables when considering feeding efficiencies, especially at larger scales. Suggestions for industrial mass rearing and future work were made.

Keywords: Integrated biosystems, waste management, insect feed production, feed depth, food particle size, bioconversions, life history traits.

2.2 Introduction

Hermetia illucens most commonly inhabits pit latrines but can live on a wide variety of feeds including manure, carrion and rotting vegetables or fruits (James 1960, 1981; May 1961; Sheppard *et al.* 2002; Tomberlin *et al.* 2002). Due to its plasticity of feed and feed conversion efficiencies (Zhou *et al.* 2013), it has recently been the subject of great research interest as a waste control agent (Gabler 2014; Kortelainen et al. 2014; Manangkot et al. 2014; Pieterse et al. 2014; Stamer et al. 2014; Widjastuti et al. 2014; Tschirner et al. 2016); Most notably is its' ability to reduce organic wastes by about 60% through its larval (or feeding) stage (Newton *et al.* 1977; Bondari & Sheppard 1981; Sheppard *et al.* 2002; Barry 2004; Gabler 2014). In this process, *H. illucens* can bioconvert organic waste into a valuable source of protein and lipids (Hale 1973; Newton *et al.* 1977; Bondari & Sheppard 1981; St-Hilaire *et al.* 2007a, 2007b; Banks 2008).

Many entrepreneurs have sought to mass-produce these flies to benefit from their advantageous traits (Drew & Pieterse 2015). However, to achieve marketable products (i.e. a lipid, chitin and protein-rich *H. illucens* feedstuff and fertiliser amendment), it is essential to ensure that larvae can feed readily on what they are given to optimize the duration of development, mortality, larval size and their waste reduction abilities. Larde (1989) has suggested that the depth of the larval feed may affect some of their life history traits and limit feed accessibility due to zones being formed by abiotic factors such as temperature and oxygenation of the feed. However, this study is specific to *H. illucens*' performance in one type of uncommon feed (i.e. coffee pulp) under natural conditions and therefore is limited in a commercial context where all factors would be controlled for optimal results and a mixed food waste feed would be most optimal (Nguyen et al. 2013).

An additional variable that may have significant effects would be particle size of the feed as this may influence their ability to uptake nutrients due to their poor ingestion abilities

(Schremmer 1986). Not only would large particle sizes in the food require more energy for the larvae to feed than small particles, they would potentially create variable nutrient zones that the larvae may need to move through and may cause significant variability in the size of larvae from areas of poor or varying nutrient composition. Additionally, to my knowledge, no study has addressed how particle size affects the life history of *H. illucens* larvae and therefore have included this variable in conjunction with feed depth as they may have varying effects across the combinations of treatments.

The aims of this study were to understand: (1) what effect feed depth and particle size has on the ability of *H. illucens* to reduce and bioconvert wastes, (2) what effect feed depth and particle size has on the survival, development and final larval mass (i.e. fifth larval instar) and (3) what effect feed depth and particle size has on the depth profiles of both the larval aggregations and their feed. The following alternative hypotheses have been suggested: firstly, deeper feed and smaller particle sizes cause decreases in depth profiles of larvae, final instar larval mass, development, survival, bioconversion and waste reduction; and secondly, with increasing depth and decreasing particle sizes, will force individuals into smaller aerobic zones where they can only feed thus increasing their densities and changing the dynamics of the aggregation's performance due to induced competition.

2.3 Methods and materials

2.3.1 Source of larvae

Six to twelve populations of ca. 12 000 adult *H. illucens* were reared in durable mesh enclosures (2.5 m^3) for less than six months under controlled conditions (25.94 ± 3.56 (SD) °C; 66.7 ± 12 (SD) % relative humidity). Adults were supplied with water (moist cloth held in containers) and proprietary lighting conditions (12:12h light: dark cycles) to ensure optimal mating and oviposition conditions (Sheppard et al. 2002; Zhang et al. 2010). The populations were

obtained from a ca. four-year old colony started at AgriProtein Technologies© from a locally-obtained wild population. A mesh-covered kitchen waste attractant (< 2 days old) was used in containers with proprietary grids suspended above to promote oviposition by females (Sheppard *et al.* 2002). Adults were given 24 h to oviposit until grids were removed and checked for eggs.

Hermetia illucens eggs were incubated inside an open container (5 Lt) in an AquaLytic ThermoStatic Cabinet (Model: AL655, 260L) at 27.5°C and ~90% relative humidity for 72 h (Sheppard *et al.* 2002). The grids were placed face-up over 2 kg of poultry laying mash (NOVA Brands, South Africa; > 1 cm deep, particle size 1-9 mm, moisture content 51.31 ± 0.58%) in a 60 Lt container. A hard-plastic mesh was placed between the two surfaces, to prevent fungal growth on the grids and accompanying eggs and the container was then covered using a saffron cloth (Demtex, ZA) and elastic. The container was placed 20 cm from the floor in an environmentally controlled chamber (26.6°C, 81.6% RH, 12:12 light: dark artificial light). Eggs hatched after 24 h, after which the egg grids were removed and larvae could feed undisturbed for 72 h to reduce mortality due to handling (Tomberlin *et al.* 2009; Nguyen *et al.* 2013). Next, an additional 5 kg of mixed organic wastes (University of Cape Town, South Africa; particle size 1-200 mm, moisture content 64.39%, 5 cm deep) was added and allowed to feed for a further 72 h as a second nursery phase. Thus, at the onset of experimentation, all larvae were between 6-7 days old (Tomberlin et al. 2009, 2012; Diener et al. 2009; Li et al. 2011a).

2.3.2 Experimental design

Three replicates and one control were run over two trial periods (started a week apart) for feed depths from 5-20 cm at 5 cm increments and for three different particle size treatments: 1. 0-5 mm^3, 2. < 30 mm^3, and 3. a control treatment. These three particle size treatments were

achieved by either blending waste and sieving through 5 mm plastic mesh, a 30 mm plastic mesh or was not sieved / homogenised at all. Additionally, a 500 g sample was taken and sent for proximate analysis for each trial and particle size treatment. These samples were tested using standardised methods (see *Appendix 2)* being analysed through Quantum Analytical Services laboratory (Malmesbury, SA) and the as is values were then calculated using their proportion to the moisture content as a percentage. Five gram samples were also taken for each trial and particle size treatment to be analysed for moisture content by drying in a convection heat drying oven at 60°C for 48 hours (*Appendix 2*).

Clear plastic cylindrical containers ((r) 5.6 x (H) 28.2 cm) were filled with kitchen waste (supplied by the University of Cape Town) to the allotted depth treatments, then covered with saffron cloth and elastic to prevent colonization of other insect detritivores. The containers were then placed in a Latin square design on shelving 1.5 m above the ground. It was observed that feed when left to stand would increase in depth without any manipulation or handling and that air bubbles within the feed were visibly forming. Therefore, it was deduced that gas production through fermentation of the feed was inducing changes (i.e. feed-induced fermentation) before larvae were added and it was necessary to address this so that set feed depths could be used initially when larvae were added. Feed was given 24 h to allow for fermentation-induced depth changes, after which the depths were removed to the specified treatment depths and reweighed as an initial feed supplied. Seven control replicates, lacking larvae, were also included for each treatment group to account for any possible waste reduction in the food caused by bacterial activity and/or evaporation.

Each larva was fed 89 ± 5 mg/day; as close as was possible to the optimal 100 mg/larva/day (Diener et al. 2009); over a 15-day experimental period (i.e. 21 days of feeding from hatching). The number of larvae added to each container differed between treatments so that density in each container was constant to the food provided (i.e. 1.5 g feed to a single larva). Larvae were

inoculated on the feed in each container as their starting point and depth measurements were started one day after inoculation. Thereafter, larvae were observed through the peripheries of the clear containers every two days and the maximum depths of the larvae, and the change in feed depth, were recorded from the bottom of the container throughout the experiment using a standard measuring tape. Additionally, temperatures at the top (i.e. where the aggregation occurred) and bottom (i.e. mostly feed temperature) of the feed (about 2 cm from the container walls to avoid edge effects) was recorded using a glass mercury thermometer. Ambient temperature and humidity were also recorded daily using iMonnit humidity and temperature sensors (Monnit Corp, Utah, US) and an ExTech temperature and humidity data logger (Model: RHT10; Nashua, New Hampshire) for crosschecking data produced on environmental conditions.

At the end of the experimental period, containers with all contents were weighed; a 10 g sample of mixed feed was taken and thereafter all larvae and prepupae were washed out of their feed using water and a 5 mm sieve to catch larvae and wash through feed (termed 'wet separation'). Thereafter, larvae were dried of exterior water using paper towels and cooled to 10°C to slow development without euthanizing the individuals. Prior to this, biomass of the larvae was weighed (Scale Balance; Model: Sartorius AY3101) and feed weight was calculated by subtraction of larval and container weights.

Dry mass was recorded by either drying for 48 h at 60°C or by using a moisture content meter (Chanzhou Xingyun Electronic Equipment Co., Ltd, Halogen Moisture Analyzer; Model: XY-105MW) for all larval and feed samples taken at the end of the experiment (*Appendix 2*). Provisioning ration change (PRC), waste reduction (WR), bioconversion and optimal bioconversion deficit (OBD) for all containers were calculated (*Appendix 2*).

All individuals were counted for survivorship and the number of prepupae was taken as a proxy for development. Fifteen fifth-instar larvae were selected at random from each container and

weighed individually (Kern ABJ 320-4 Electronic Balance) to get individual larval mass recordings for individual mass variation comparisons between depth treatments.

2.3.3 Statistical analyses

Power analysis was run on pilot data and data from the two trials to verify sample size ($p > 0.8$). Tests for homoscedasticity (via a Levene's tests) and normality (Distribution-fitting graphs, p-plots and Kolmogorov-Smirnoff tests) were run to confirm assumptions for parametric analysis. No percentage variables were run as parametric analyses but were completed using GLM analyses due to the lack of normality concerns with proportion data (REF). The only variables which were parametric were as follows:Time to maximum depth and maximum aggregation heat difference.

Statistical differences between the two trials were run to decide whether to pool the data or analyse separately for each variable investigated. Tests of differences between trials were also run on all environmental variables to ensure their standardised nature. All data was pooled except the following variables: provisioning ration change (%), bioconversion (% - DM), rate of feed depth accessed (% total/day), time to maximum feed depth (days) and maximum larval aggregation heat (°C).

Proximate analysis results for the feed were compared between particle size treatments and trials to check for significance between trials too. Larva-free controls were also compared between all treatments for the following variables: waste reduction (% - DM), maximum and time to maximum larval aggregation heat and rate of feed depth accessed (% total/day).

Data recorded over time during the experiment were calculated as a rate per two days to increase robustness of the analyses and change in larval aggregation heat (as a maximum for the experimental period only). Time to the maximum feed depth accessed or the maximum aggregation heat change was also calculated and analysed.

Final mass of individual fifth-instar larvae was analysed using a Repeated Measures Analysis of Variance (RMANOVA). Waste reduction, OBD, time to maximum aggregation heat change and rate of feed lost per day were analysed between depths and particle sizes using General Linear Models (GLM). All other variables were analysed using a Multivariate Analysis of Variance (MANOVA). Pearson's correlations were calculated to assess any relations between all main variables, especially with respect to the theoretical values of OBD and PRC with feed depth accessed and other feeding efficiency variables. All analyses were analysed in Statistica (StatSoft 2007).

2.4 Results

2.4.1 Larva-free controls and feed composition

Larva-free controls showed significant differences from larvae treatments for all variables investigated for waste reduction (GLM: $F = 321.93$, d.f. = 1, $p << 0.001$). Values were highest for waste reduction at the blended 5 cm trials but averaged less than 26% waste reduction. Feed depth lost per day (% total/day) was also not more than 5% lost per day with no significance between any variables (GLM: $F = 0.2451$, d.f. = 6, $p > 0.05$). Maximum feed heat was also non-significant with values not exceeding 1°C for almost all treatments across trials taking, at most, 14 days to reach maximum temperature.

The feed composition within trials was mostly consistent across treatments in both trials (Table 2.1). Statistical analysis showed no significant differences between either trials or particle sizes for all the nutrient values.

2.4.2 Larval aggregation heat

A summary of the main variable significances and their interactions show high significance for diet depth and particle size and for some of their interactions depending on the variables (Table

2.2). Average time to maximum larval aggregation heat varied from 1-7 days between treatments. Larvae in blended feed at 5 cm depths took the longest to reach their maximum temperature while 15 cm depths of blended feed took the least amount of time. Statistical analysis showed that depth had a significant effect (GLM: $F = 19.50$, d.f. = 3, $p << 0.001$), while there was no significance for particle size treatment or the interaction effect between them with pooled data. Feed depths at 5 cm were most significant from the rest of the feed depth treatments being the slowest to reach maximum temperatures (Tukeys: $F = 19.50$, d.f. = 3, $p < 0.001$).

Maximum larval aggregation temperature showed significant differences between trials (MANOVA: $F = 7.32$, d.f. = 1, $p = 0.0095$) and therefore trial was included in the analysis as an additional variable. Temperatures were consistently higher above 5 cm depth treatments but highest with blended feed at 15 cm reaching a maximum increase from ambient of between 5-6°C. There was significance between both feed depth (MANOVA: $F = 27.87$, d.f. = 3, $p << 0.0001$) and particle size treatments (MANOVA: $F = 3.852$, d.f. = 2, $p = 0.028$) and for the interaction effect between the two variables (MANOVA: $F = 3.54$, d.f. = 6, $p = 0.0056$). Statistical significance was seen between blended and sieved particle size treatments (Tukeys: $F = 3.852$, d.f. = 2, $p = 0.022$) where blended was generally lower than sieved. Feed depths showed significance between almost all the 5 cm treatments and all other treatments (Tukeys: $F = 27.87$, d.f. = 3, $p = 0.000167$) where they were colder. Treatments of 5 cm depths for control feeds showed no significance (Tukeys: $F = 3.54$, d.f. = 6, $p > 0.8$) in most cases when interaction effects were looked at while blended feeds of 15 cm depths showed the opposite with most other treatments (Tukeys: $F = 3.54$, d.f. = 6, $p < 0.02$) being significantly higher.

2.4.3 Feed accessed

Feed accessed varied across treatment, ranging from 35-100% (Figure 2.5). There were significant differences for maximum feed accessed within feed depth (GLM: $F = 144.91$, d.f. $= 3$, $p = 0.025$) and particle size (GLM: $F = 3.90$, d.f. $= 2$, $p << 0.0001$). Larvae access blended feeds significantly better when compared to other particle sizes (Tukeys: $F = 3.90$, d.f. $= 2$, $p = 0.027$); specifically, feed depths of 15 and 20 cm were significantly lower (Tukeys: $F = 144.91$, d.f. $= 3$, $p < 0.001$) than 5 and 10 cm, which were not significant from each other (Figure 2.1).

There were significant differences in the rate of feed accessed between feed depth (GLM: $F = 43.01$, d.f. $= 3$, $p << 0.001$), particle size (GLM: $F = 3.46$, d.f. $= 2$, $p = 0.039$) and the interaction effect (GLM: $F = 4.39$, d.f. $= 6$, $p = 0.0013$). Feed depths of 5 cm were significantly higher

than all other treatments (Tukeys: F = 43.01, d.f. = 3, p = 0.00017) but also more variable (

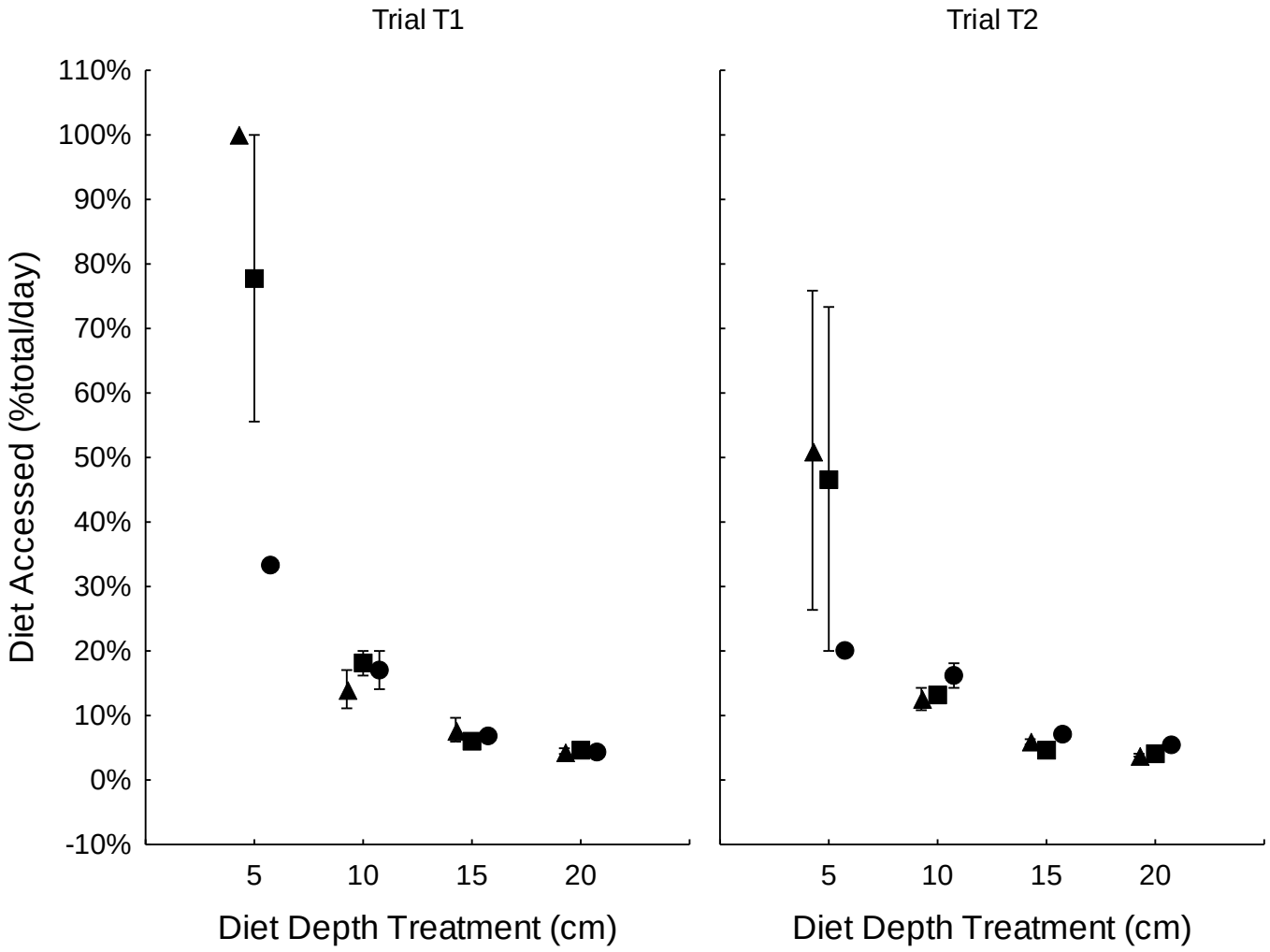

Figure 2.6). Particle size treatments were only significantly different between blended and control treatments (Tukeys: F = 3.46, d.f. = 2, p = 0.037). Blended 5 cm treatments were significant to the other 5 cm treatments only. Sieved and control 5 cm treatments were not significantly different between each other when interaction effects were considered (Tukeys: F = 4.39, d.f. = 6, p = 0.93).

Time to access maximum feed also showed significance between feed depths (MANOVA: F = 129.28, d.f. = 3, p << 0.001), particle sizes (MANOVA: F = 3.67, d.f. = 2, p << 0.001) and interaction effects (MANOVA: F = 4.11, d.f. = 6, p = 0.0021). Feed depth treatments were significantly different (Tukeys: F = 129.28, d.f. = 3, p < 0.001) except for 15 and 20 cm

treatments and all others, which were not significant (Tukeys: F = 129.28, d.f. = 3, p > 0.9). Particle size was significant only between sieved and controlled treatments (Tukeys: F = 3.67, d.f. = 2, p = 0.035).

2.4.4 Survival and prepupae

Survival showed a negative trend from 5 – 20 cm treatments and between particle sizes (Figure 2.7). Significant differences occurred between feed depth (GLM: F = 188.56, d.f. = 3, p << 0.001), particle sizes (GLM: F = 18.10, d.f. = 2, p << 0.001) and their interaction effect (GLM: F = 3.26, d.f. = 6, p = 0.0076). Particle sizes showed significance between blended and other treatments only (Tukeys: F = 18.10, d.f. = 2, p < 0.0001) while feed depth showed significance between 15 and 20 cm treatments with all other treatments (Tukeys: F = 188.56, d.f. = 3, p < 0.0001) while 5 and 10 cm treatments showed no significance within each particle size treatment.

The number of prepupae showed major differences between 5 cm trials and all other feed depths (Figure 2.8). Statistically, there was only significance between feed depth treatments (GLM: F = 547.38, d.f. = 3, p << 0.001) but not between particle sizes or the interaction effects. Particularly, 5 cm treatments were highly significant to all other feed depth treatments (Tukeys: F = 547.38, d.f. = 3, p = 0.000156) but not between any other treatments.

2.4.5 Feeding efficiencies

Waste reduction showed a negative trend for all treatments (Figure 2.9). There was no significance for the interaction effects between feed depths (GLM: F = 29.27, d.f. = 3, p << 0.001) and particle sizes (GLM: F = 133.51, d.f. = 2, p << 0.001). Sieved particle sizes showed significantly lower averages than all other particle sizes (Tukeys: F = 133.51, d.f. = 2, p = 0.000117). Feed depths showed significance between all depths (Tukeys: F = 29.27, d.f. = 3, p < 0.01). Significant correlations were also found between waste reduction and the following

variables: survival (r = 0.79), number of prepupae (r = 0.73) and maximum feed depth accessed (r = 0.78).

Bioconversion showed similar trends to waste reduction but with less variance in the error with exception of Trial 2, blended 10 cm treatment, which had significant variance (Figure 2.10). Trials showed significance (GLM: F = 7.51, d.f. = 1, p = 0.0086) and so did feed depths (GLM: F = 95.84, d.f. = 3, p < 0.001) and particle sizes (GLM: F = 12.19, d.f. = 2, p << 0.001) but interaction effects were only significant between particle sizes and trial for interaction effects (GLM: F = 3.25, d.f. = 2, p = 0.047). All feed depths were significant (Tukeys: F = 95.84, d.f. = 3, p < 0.003) and blended particle sizes did show significant differences with all other treatments (Tukeys: F = 12.19, d.f. = 2, p < 0.003). A strong correlation was additionally found between bioconversion and survival (r = 0.87), and between bioconversion and maximum feed accessed (r = 0.78).

Provisioning ration changes (PRC) ranged from 0.31 to 231 mg/larva/day across all treatments. Significance was seen between trials (GLM: F = 5.079, d.f. 1, p = 0.029), particle sizes (GLM: F = 16.0014, d.f. = 2, p << 0.001), feed depths (GLM: F = 115.87, d.f. = 3, p < 0.001), interaction effects between trial and feed depths (GLM: p < 0.001, d.f. = 3, F = 8.55) and between feed depth and particle sizes (GLM: F = 6.51, d.f. = 6, p < 0.0001). Particle sizes showed significance between blended particle sizes and all other treatments (Tukeys: F = 16.0014, d.f. = 2, p < 0.0002). Feed depths of 15 and 20 cm depths only showed significance between all other trials and themselves (Tukeys: F = 115.87, d.f. = 3, p << 0.001). Exceptions were seen in the interaction effects where values were non-significant for sieved 15 cm and all other treatments except the aforementioned and 20 cm treatments (Tukeys: F = 6.51, d.f. = 6, p < 0.001). No significant differences were seen between sieved and control 15 cm depths (Tukeys: F = 6.51, d.f. = 6, p = 0.95), blended 20 cm and control 15 cm (Tukeys: F = 6.51, d.f. = 6, p = 0.88), and significance was seen between sieved 20 and 5 cm sieved treatments

(Tukeys: F = 6.51, d.f. = 6, p < 0.04). Correlations were additionally seen to be significant between PRC and survival (r = - 0.95), waste reduction (r = - 0.69) and bioconversion (r = - 0.78).

Optimal bioconversion deficit (OBD) showed a similar trend to that of survival (Figure 2.11). Statistical significance was found between feed depths (GLM: F = 161.42, d.f. = 3, p << 0.001), particle sizes (GLM: F = 17.63, d.f. = 2, p << 0.001) and the interaction effect (GLM: F = 3.46, d.f. = 6, p = 0.0053). Blended particle sizes showed significant differences from other particle sizes (Tukeys: F = 17.63, d.f. = 2, p << 0.001). Feed depths also showed differences for 15 and 20 cm treatments and all other treatments (Tukeys: F = 161.42, d.f. = 3, p << 0.001) whereas 5 and 10 cm treatments showed no significance. Interaction effects showed no significance for sieved and control 15 and 20 cm treatments as exceptions to the aforementioned (Tukeys: F = 3.46, d.f. = 6, p > 0.05). Correlations were also seen significantly between OBD and the following variables: survival (r = 0.997), waste reduction (r = 0.801) and PRC (r = - 0.95).

2.4.6 Larval mass

Larval mass decreased from highest averages in the 5 cm treatments to the lowest in the 20 cm treatments (Figure 2.12). Statistical significance was seen for both particle size (RMANOVA: F = 1.72, effect d.f. = 30, p = 0.0196) and feed depths (RMANOVA: F = 2.34, effect d.f. = 45, p = 0.000037) but no significance was found for interaction effects (RMANOVA: F = 0.85, effect d.f. = 90, p > 0.05). Further statistical significance between treatments could not be seen for particle sizes (Tukeys: F = 1.72, effect d.f. = 30, p > 0.05) and feed depths of 5 cm only showed significance from 15 and 20 cm in feed depth treatments (Tukeys: F = 2.34, effect d.f. = 45, p << 0.01).

2.5 Discussion

2.5.1 Larva-free control and feed composition

Larva-free controls showed that, in the absence of larvae, waste reduction was still observed, as there are also inputs from the feed itself which caused a change when larvae were not present. It is possible that the reduction of feed and depth may be caused by a loss of water via evaporation or bacterial activity (via metabolic expenditure).

Feed composition showed comparable values to those in other trials (Tomberlin et al. 2002; Ojeda-Avila et al. 2003; Gobbi et al. 2013; Nguyen et al. 2013; Oonincx et al. 2015). A good balance of all nutrients across treatments made a good feed as the larvae responded well in terms of their feeding efficiencies. Because of the variable feed sources over the two trial periods, it was surprising that proteins and lipids did not vary between the trials as much as in the fibre contents, even if not significantly. The role of fibre in *H. illucens* feeding merits further research. Moisture content of the feed was found to be above average (i.e. 60%) but fell within previously-investigated moisture contents that were suitable for optimal feeding of the larvae (Fatchurochim *et al.* 1989). However, a major concern is the crude fat analysis method which does not accurately measure fat in insects (see *Appendix 2, crude fat*). It is, therefore, suggested that the Method used by Quantum should be replaced with a more modern method for measuring insect crude fat more efficiently. Additionally, protein content doesn't not take into account Nitrogen fixed Extracts (NFEs), this may be necessary to understand the intricacies of nutrient assimilation for these larvae in future studies.

2.5.2 Larval aggregation heat

Larval aggregation heat, often called maggot-generated heat, has been shown to occur in many dipteran larval masses (Donovan *et al.* 2006; Slone & Gruner 2007). Additionally, metabolic activity is closely linked with this heat generation *en masse* (Slone & Gruner 2007). In this

study, *H. illucens* were most metabolically active during their first week of feeding and generally decrease thereafter. Additionally, their immediate environment increased only as high as 6°C from their optimal ambient temperatures (Tomberlin et al. 2009; Harnden & Tomberlin 2016; Holmes et al. 2016). This is interesting because other studies (Donovan *et al.* 2006; Slone & Gruner 2007) have reported significant heat increases from dipteran masses to as much as 20°C larval aggregation heat. This is possibly a mechanism that allows maggots to survive fluctuating conditions and continue development regardless of the environment. Further studies could aim to understand how *H. illucens* varies in their feeding abilities (feed access included) when ambient temperatures are suboptimal, as this may also affect how they can take in nutrients (House 1961).

2.5.3 Feed accessed

Feed depth is a novel concept to *H. illucens* life history but, from these results, seems to be an important link to feed accessibility. While duration, rate and feed accessed all show significance between feed depth and particle size treatments, the applicable value is seen most prominently in feed accessed and duration. Larvae in the 5 and 10 cm treatments could access feed fastest and were the only larvae able to access the full amount provided. This becomes intrinsically linked to density and feeding ration, as these will change if larvae cannot access the allotted ration (Stiling 1988; Green et al. 2003; Diener et al. 2009). Varying feed depths, below 5 cm (Sheppard et al. 2002; Tomberlin et al. 2009; Diener et al. 2009; Nguyen et al. 2013) to about 20 cm (Lardé 1989, 1990), have shown similar results and while depths of 5 cm are adequate for study purposes, upscaling may require a greater understanding of feed accessibility when constructing rearing environments (Kroes 2012). Additionally, many studies remove feed and replace daily (Sheppard *et al.* 2002; Tomberlin *et al.* 2009; Yu *et al.* 2011; Nguyen *et al.* 2013), which alone may have effects on larval provisioning rations if depth is not shallower than 5 cm, and is something to consider when replacing food in future trials.

What is abundantly clear is that using 15 and 20 cm feeds will impact on feeding efficiencies by limiting feed accessibility and increasing larval density.

However, what was interesting is that blended particle size treatments in some feed depths caused lower rates of access. This may be because of the highly viscous nature of the blended feed, which made movement by larvae more intense, and also less oxygen available at depth, therefore slowing down their ability to access feed. What is unclear, and needs further investigation, is if larvae are able to actively, suspend respiration in order to reach lower depths of food available or not. If this is the case, how long will this period be before it is fatal for the larvae?

This, considering feed composition, brings up the question of the variable nature of waste streams, especially over many different geographical locations (Taljaard *et al.* 2005; Diener *et al.* 2009, 2011; Nahman *et al.* 2012) and the variable feeding plasticity of *H. illucens* (Zhou *et al.* 2013). It is advised that more trials be conducted over larger temporal and spatial scales to determine if changes in feeding plasticity also cause changes in feed accessibility that could not be distinguished over two trial periods in one colony of *H. illucens.*

2.5.4 Survival and prepupae

Survival may be of major importance when upscaling production of larvae therefore it is essential to increase the number of individuals that complete the feeding period given to them. This study has shown that larvae survive best when kept below 10 cm with blended feeds or with any particle size at 10 cm feed depths, thus linking the importance of particle size to survival of *H. illucens* larvae. Additionally, this paper has shown that survival will inevitably effect bioconversion and waste reduction, thus allowing larger individuals in larger quantities for product harvesting when fed blended feed under 5 cm deep.

Prepupation numbers show the relative development rates of the larvae since the experiment had a fixed time variable. This is useful as most studies look to measure time to prepupation (Lord *et al.* 1994; Amatya 2009; Tomberlin *et al.* 2009; Zheng *et al.* 2012; Bonso 2013) but under industrial conditions it may be necessary to include deadlines for feeding times and harvesting times to allow for stable state production. Treatments of 5 cm always had significantly higher numbers of prepupae, showing that the days to reach largest final white larvae (being the largest individuals with the most biomass to harvest (Gobbi 2012)) were probably significantly shorter due to the change in feed depths.

2.5.5 Feeding efficiencies

Waste reduction showed a linear trend for feed depth treatments which could be associated with feed depth and survival of the larvae as shown in the correlation results. Therefore, it is necessary to control feed depths to control or increase waste reductions. In terms of particle size effects, it was seen that sieved particle sizes caused decreased waste reductions over control treatments which was surprising. This merits further research to justify why this may have occurred. Blended feeds always had the best waste reductions and therefore it is recommended that industry should blend their feeds (or rather reduce their particle size) if they would like increased waste reduction ability of the larvae with the optimum being at 5 cm depths.

Bioconversion values, while varying between trials, showed similar trends between treatments except for a single extreme value which caused major variation in one treatment. This value was an outlier that was exaggerated due to number of replicates in the trials but identifying its cause would require further experimentation. Bioconversion seems to be an effective and simple method for calculating feeding efficiencies of the larvae for industrial reasons but has limited use in the literature (Hem *et al.* 2008; Bonso 2013; Zhou *et al.* 2013; Banks *et al.* 2014;

Leong *et al.* 2015; Li *et al.* 2015; Paz *et al.* 2015; Surendra *et al.* 2016). Like waste reduction, bioconversion is greatly affected by survival and feed accessibility and therefore it is recommended that treatments be blended and kept below 5 cm for improved bioconversion when upscaling larval mass rearing.

The use of PRC and OBD have significance in future research as they give an idea of how nutrients are being used in feed (due to mortality and accessibility) and by showing the deficit from optimal bioconversion that an environment is causing (primarily due to mortality and larval mass variability), respectively. While both are novel and unique ways of looking at feeding efficiencies, they both provide viewpoints which are simple and time-saving ways of assessing larval performance in a mass rearing colony. To further enhance these two formulae for future use, it will be necessary to test their use stringently under varying conditions and confirm, through mathematical modelling, that they are viable calculations.

2.5.6 Larval mass

Larval mass showed a strong trend with bioconversion and waste reduction but has its own merits as it gives a more individual look at how larvae performed compared to the population approach that feeding efficiencies use. It is seen that larval mass trends negatively as feed depth increases and this may be due to overcrowding stress not allowing the larvae to metabolise as the access to feed and the space they could feed in changes dramatically (Conde & Rabinovich 1979; Applebaum & Heifetz 1999; Hooper *et al.* 2003). Other literature has looked at how competitive stress can negatively affect insects but none have investigated this on an industrial scale (Applebaum & Heifetz 1999; Green *et al.* 2003; Hooper *et al.* 2003; Paz *et al.* 2015). In many ways, the feeding rates of Diener *et al.* (2009b) is a measure of density and could therefore be used to further test how competition on a broader spectrum could affect *H. illucens* larvae. Additionally, larvae could take up more nutrients in the blended feed trial; and therefore,

become larger individuals due to the greater digestibility of that feed. It is therefore recommended that the larvae be fed blended feed at less than 5 cm depth to harvest the largest individuals possible.

In conclusion, this paper has provided a solid foundation for understanding how feed depth and particle size effect accessibility of the feed and recommends that when upscaling for industrial mass rearing purposes that the feed depths always be kept below 10 cm for full feed accessibility but will require lower than 5 cm for faster and larger larval development and optimal feeding efficiencies. Future research may need to focus upon how larvae change this feed access over spatial and temporal scales. Finally, it may be essential to see how this access changes when changing the number of individuals in a population compared to just feeding density as this may influence feeding efficiencies and how feeding densities will affect larval feeding efficiencies and accessibility.

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2.7 Table

Table 2.1: Initial nutritional composition for feed at all particle size treatments over two trial periods for feed presented to black soldier fly larvae, *Hermetia illucens* over a 15-day period. Values are based on dry matter analysis and values are in percentages with no significant differences between any of the trials or treatments.

TRIAL	PART. SIZE TREATMENT	CRUDE PROTEIN	ASH CONTENT	LIPIDS	CRUDE FIBRE	MOISTURE CONTENT	CARBOHYDRATES
T1	**Control**	11.54	2.42	11.73	0.85	69.04	18.29
T1	**Sieved**	5.49	1.54	7.24	0.58	78.16	13.10
T1	**Blended**	10.50	2.49	11.81	5.50	67.59	17.65
T2	**Control**	14.39	3.48	12.64	3.44	61.70	28.12
T2	**Sieved**	10.83	2.64	9.40	1.91	68.51	21.18
T2	**Blended**	8.95	2.39	7.90	1.09	71.42	19.69

Table 2.2: Summary table of significance and interaction effects for all variables in terms of the treatments and their interactions (p>0.05). Sig. = significance (>0.05), n.s = not significant (<0.05).

		SIGNIFICANCE (P>0.05)				
Variable	**n**	**Trial (T)**	**Depth (D)**	**Particle Size (PS)**	**Interaction**	**Type**
Survival	6	-	Sig.	Sig.	Sig.	D-PS
Prepupae	6	-	Sig.	n.s	n.s	-
Bioconversion	3	Sig.	Sig.	Sig.	Sig.	T-PS
Waste Reduction	6	-	Sig.	Sig.	n.s	-
OBD	6	-	Sig.	Sig.	Sig.	D-PS
PRC	6	Sig.	Sig.	Sig.	Sig.	D-PS, T-D
Max Diet Depth	6	-	Sig.	Sig.	n.s	-
Rate of diet Access	3	Sig.	Sig.	Sig.	Sig.	T-D, D-PS
Diet lost	6	-	Sig.	Sig.	n.s	-

Max Aggr. Heat Diff	3	Sig.	Sig.	Sig.	Sig.	D-PS
Time to Max Aggr. Heat Diff.	6	-	Sig.	n.s	n.s	-
Mass gained	3	Sig.	Sig.	Sig.	Sig.	T-PS, T-D, D-PS, T-D-PS
Larval Mass	3	n.s	Sig.	Sig.	n.s	

2.8 Figures

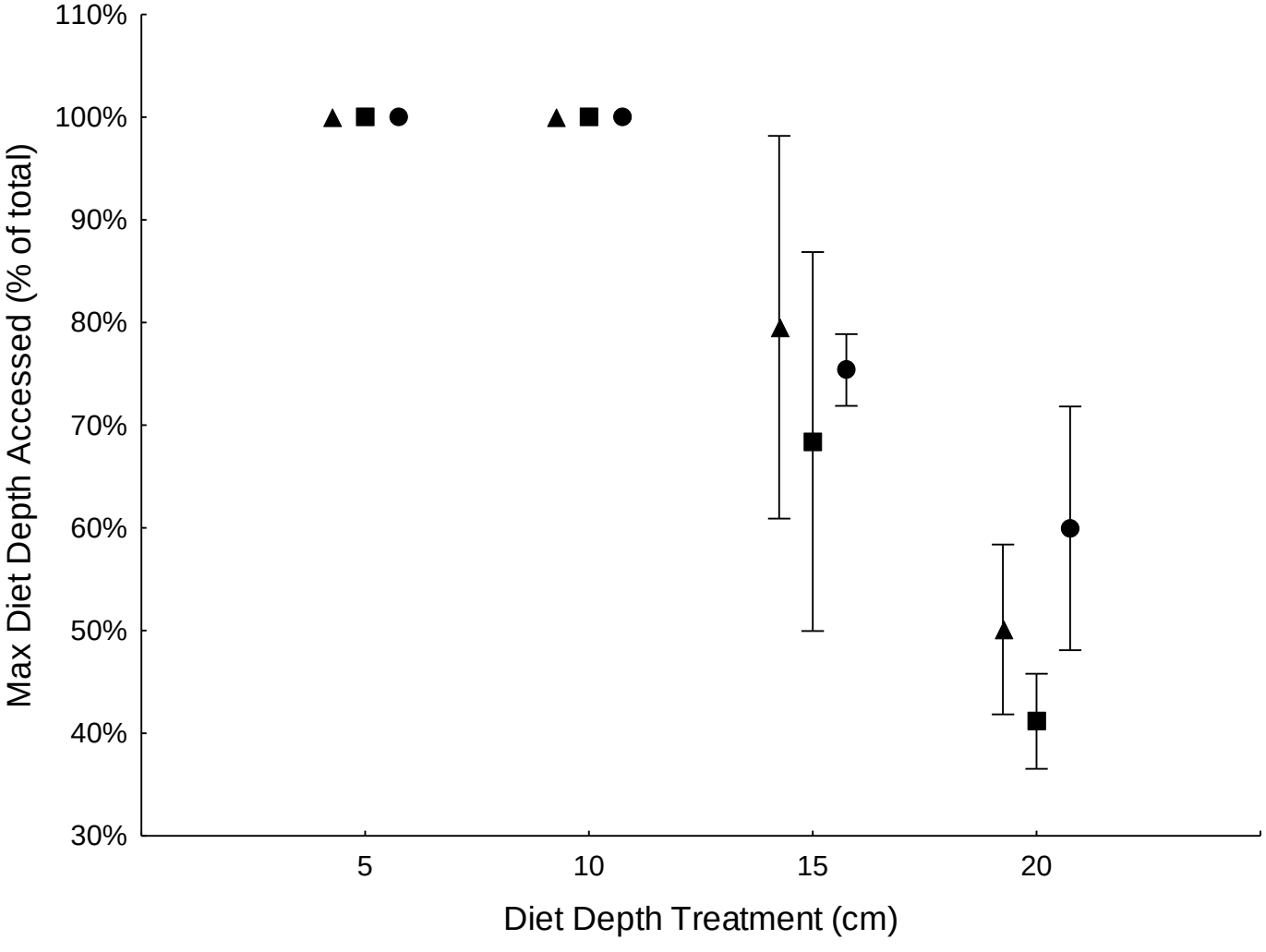

Figure 2.5: Pooled data for the maximum feed depth accessed (% of total) by black soldier fly, *Hermetia illucens*, larvae for a control (triangles), sieved (square) and blended (circular) particle size treatments along a feed depth treatment gradient over 15-day trial periods. Error bars denote 95% confidence intervals.

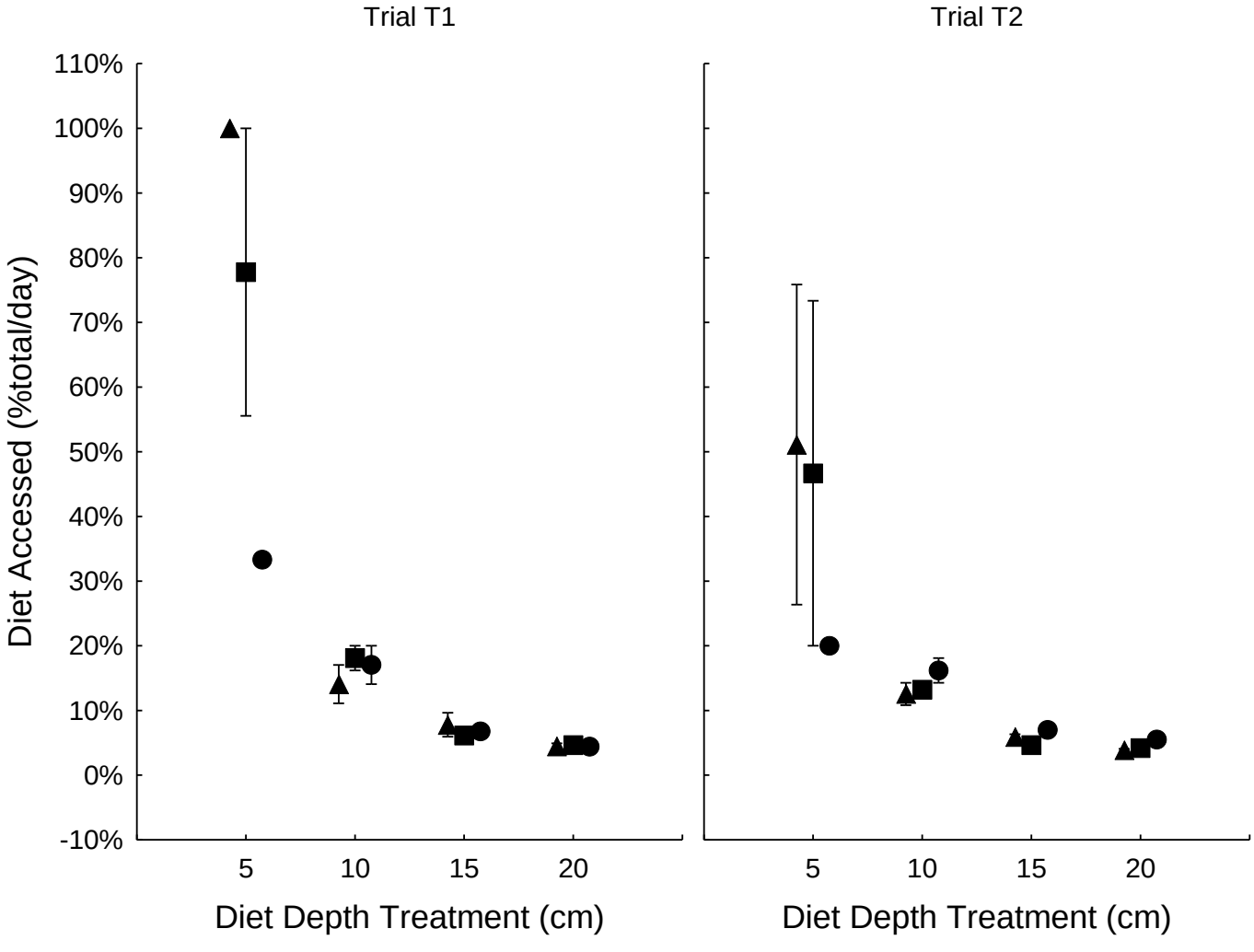

Figure 2.6: Rate of feed accessed (% of total depth/day) for a control (triangles), sieved (square) and blended (circular) particle size treatments along a feed depth treatment gradient for feeding black soldier fly larvae, *Hermetia illucens,* over two different 15-day trial periods. Error bars denote 95% confidence intervals.

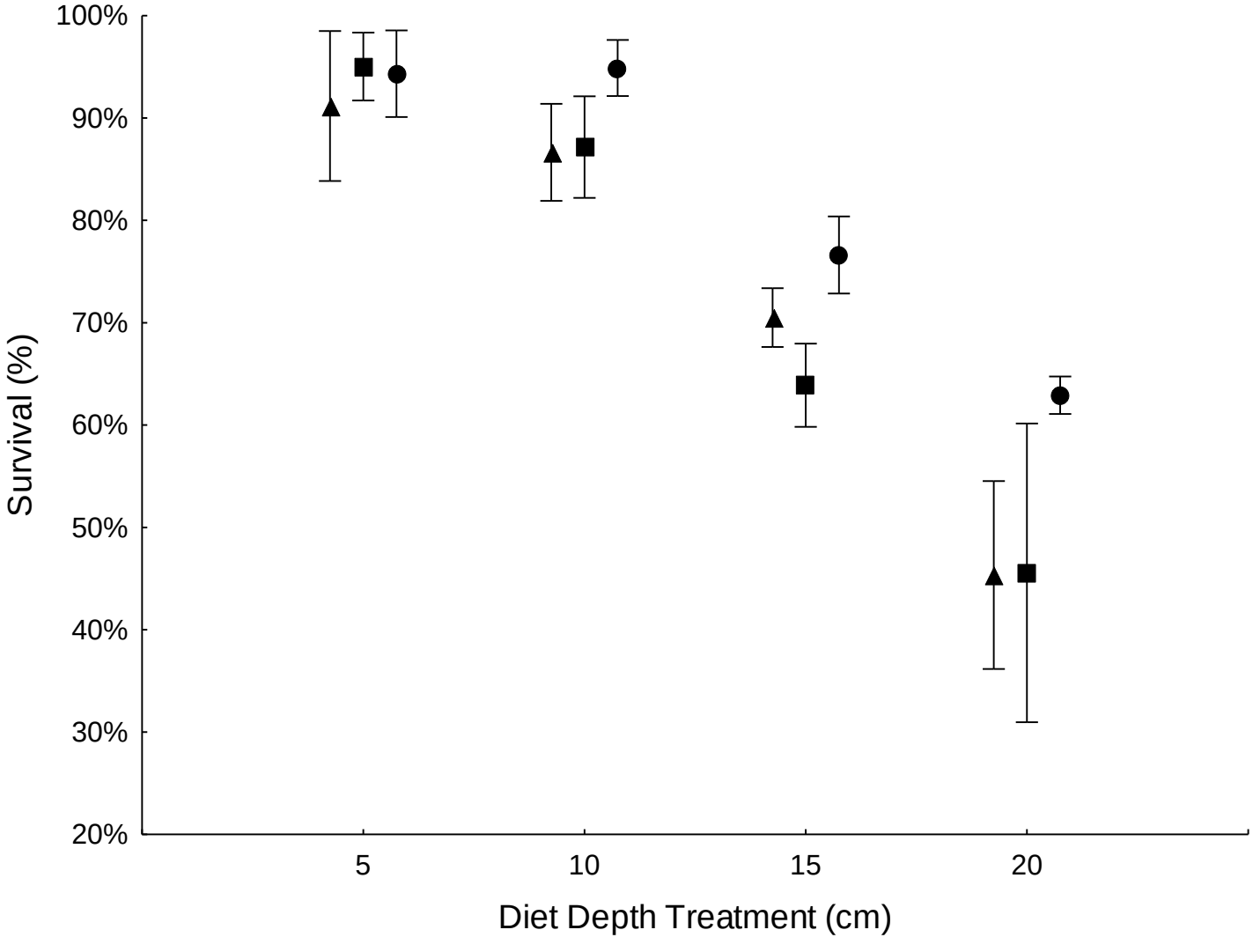

Figure 2.7: Pooled data for the survival (%) for a control (triangles), sieved (square) and blended (circular) particle size treatments along a feed depth treatment gradient for feeding black soldier fly larvae, *Hermetia illucens*. Error bars denote 95% confidence intervals.

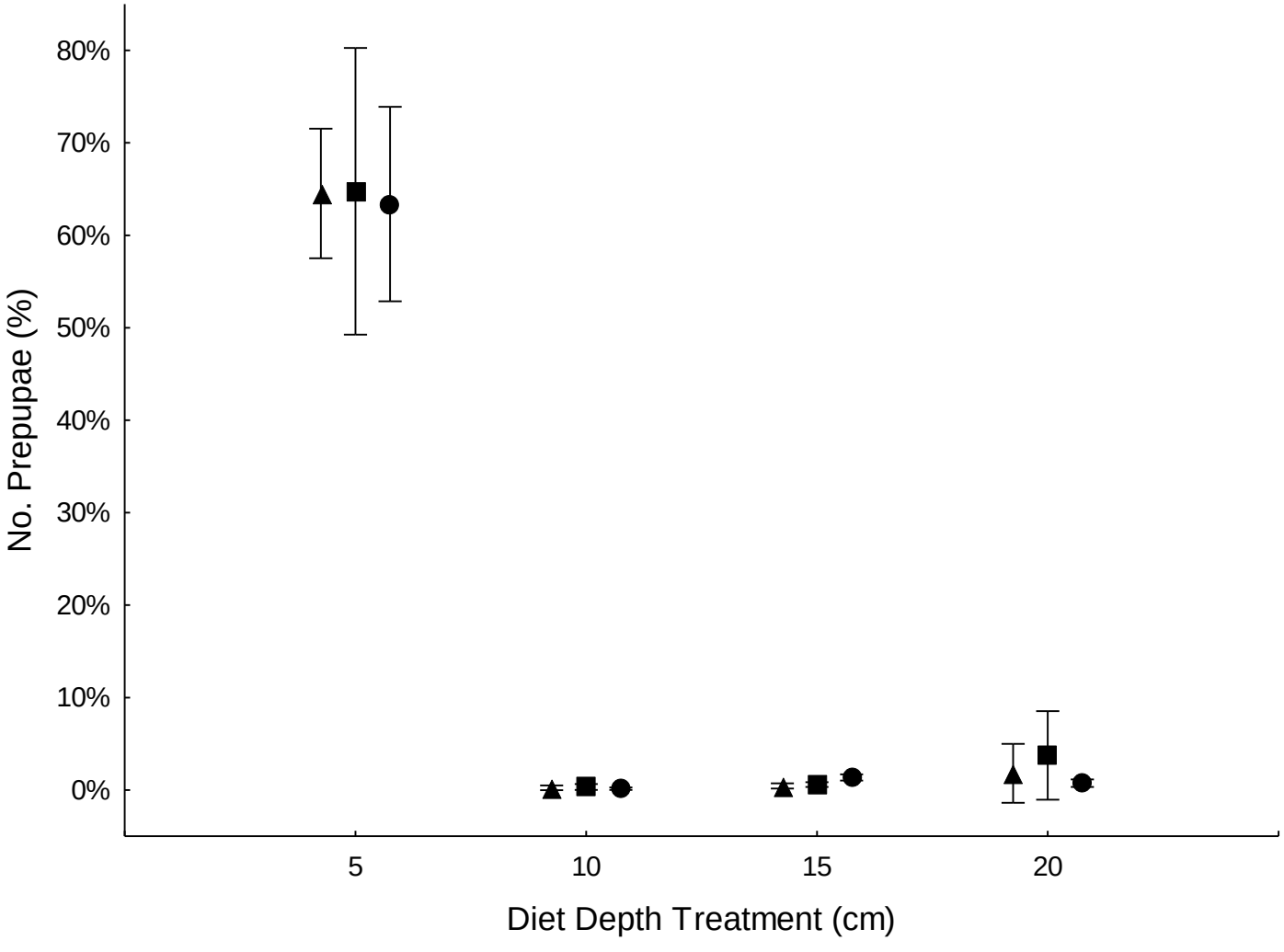

Figure 2.8: Pooled data for the number of prepupae (%) for a control (triangles), sieved (square) and blended (circular) particle size treatments along a feed depth treatment gradient for feeding black soldier fly larvae, *Hermetia illucens*. Error bars denote 95% confidence intervals.

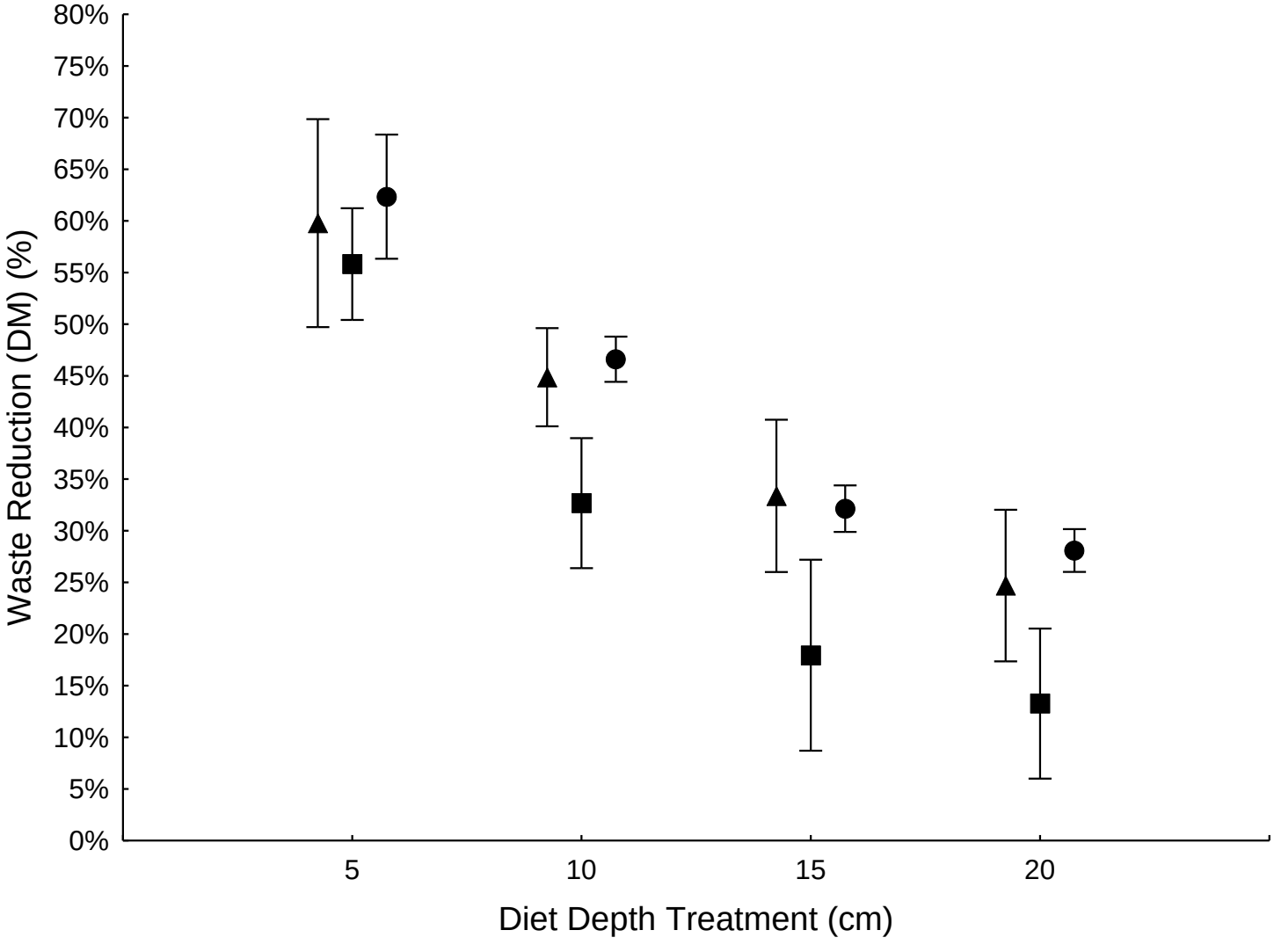

Figure 2.9: Pooled data for waste reduction (% - DM) for a control (triangles), sieved (square) and blended (circular) particle size treatments along a feed depth treatment gradient for feeding black soldier fly larvae, *Hermetia illucens,* over two different 15-day trial periods. Error bars denote 95% confidence intervals.

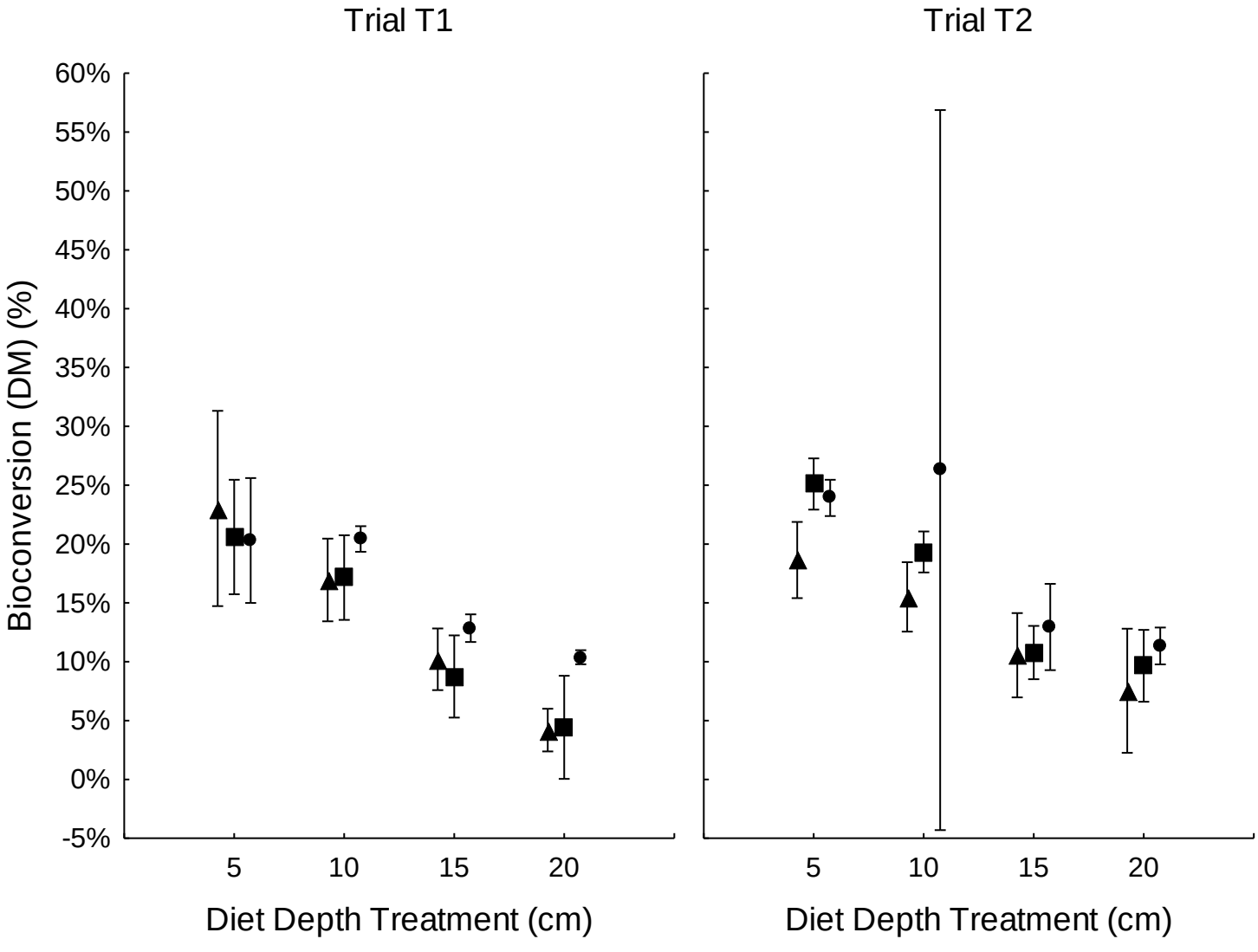

Figure 2.10: Bioconversion (% - DM) for a control (triangles), sieved (square) and blended (circular) particle size treatments along a feed depth treatment gradient for feeding black soldier fly larvae, *Hermetia illucens,* over two different 15-day trial periods. Error bars denote 95% confidence intervals.

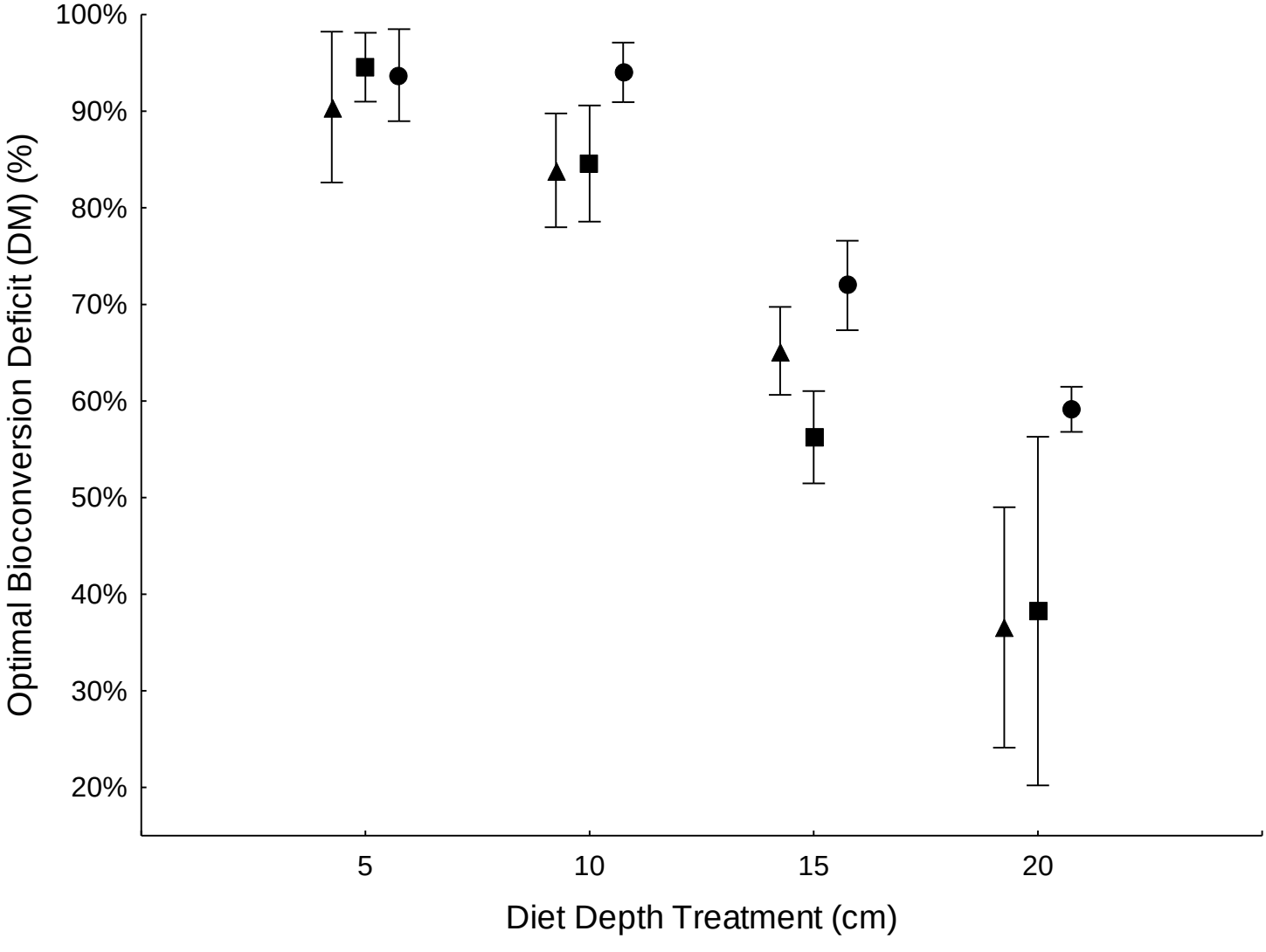

Figure 2.11: Pooled data for optimal bioconversion deficit (% - DM) for a control (triangles), sieved (square) and blended (circular) particle size treatments along a feed depth treatment gradient for feeding black soldier fly larvae, *Hermetia illucens*, over two different 15-day trial periods. Error bars denote 95% confidence intervals.

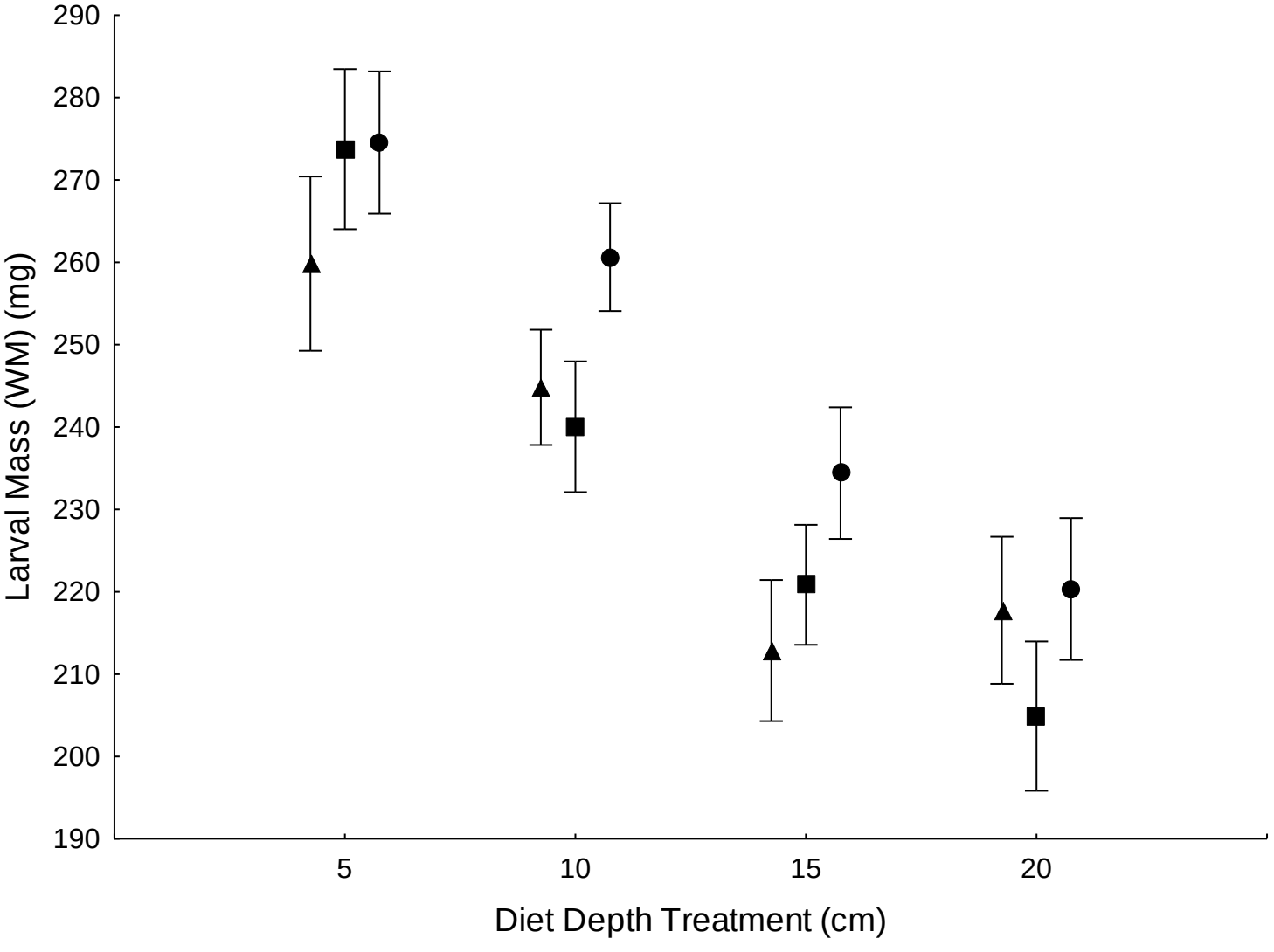

Figure 2.12: Pooled data for larval mass (mg – WM) for a control (triangles), sieved (square) and blended (circular) particle size treatments along a feed depth treatment gradient for feeding black soldier fly larvae, *Hermetia illucens,* over a 15-day trial period. Error bars denote 95% confidence intervals.

CHAPTER 3:

PROVISIONING RATION

3. The effect of larval density on a South African strain of the black soldier fly larvae, *Hermetia illucens* (L., 1758) (Diptera: Stratiomyidae) under mass-rearing conditions

3.1 Abstract

Little is known of how to feed these flies on a massive scale. This study examines what effects provisioning rations (mg/larva/day) have on varying larval densities and their feeding efficiencies using treatments between 75-150 and 200 mg/larva/day at set depths and feed particle sizes. Factors such as feed and larval proximate composition, survival, prepupation rates, bioconversion, efficiencies of conversion of digested feed, fifth instar larval mass, biomass gain, provisioning and bioconversion changes, feed accessibility and aggregation temperatures were investigated at set environmental conditions over a 15-day period on 6-day-old larvae. Larvae fed on catering waste showed significant differences in most feeding efficiency variables at densities above 100 mg/larva/day, and positive trends in larval mass, prepupation rates and survival rates with increasing provisioning rations. Larvae could access feed within the first week and aggregations were at their hottest within the first three days of experimentation. New industry-specific calculations (i.e. provisioning ration change and optimal bioconversion deficit) are discussed, along with the potential effects of intraspecific competition and geographic plasticity of larvae of *H. illucens*. Finally, suggestions are made for optimal product creation using feeding densities and the results shown here.
Key words: Integrated biosystems, waste management, commercial mass rearing, feeding efficiency, life history traits, feeding rates, biotechnology

3.2 Introduction

Current livestock proteins and lipids are becoming increasingly economically and environmentally expensive (Yaqub 1997; Sheppard et al. 2007; Merino et al. 2012; Barroso et al. 2014) and unsustainable due to overexploitation of the base resource (usually marine fish: FAO 2011; Merino et al. 2012; Thurstan & Roberts 2014; Tomberlin et al. 2015). This, coupled with issues of greenhouse gas emissions from the decomposition of organic waste in landfills, and an increasing human population (FAO 2011; van Huis et al. 2013; Alooh 2015), makes the use of an alternative feed source increasingly urgent. Therefore, the last decade has given rise to several mass-rearing companies and scientific literature (Chapter 1:; Sheppard 2002; Sheppard et al. 2007; Diener et al. 2011b; Erens et al. 2012; Kroes 2012; Makkar et al. 2014; Oonincx et al. 2015a; Drew & Pieterse 2015). However, industrialising the mass rearing process is challenging (Rumpold & Schlüter 2013; Devic *et al.* 2014), thus a better understanding of the limitations and requirements for reproduction in adult *H. illucens*, and the development and behaviour of all life stages is needed to advance mass rearing on an industrial scale.

Some environmental factors affect the life history of *H. illucens*. For instance, larvae need greater than 40% feed moisture content to survive, and at least 60% to thrive (Fatchurochim *et al.* 1989). Feed should not have particle sizes above 5 mm or depths above 10 cm (Chapter 2:) as this may limit feed accessibility and digestibility, therefore increasing larval competition and mortality (Lardé 1989, 1990).

In terms of nutrition, larvae are thought to need protein levels lower than ca. 30% and have been suggested to be better at bioconverting plant proteins than animal proteins (Arief et al. 2012; Nguyen et al. 2013). Fat and carbohydrate requirements should be similar to, or lower than, protein levels, suggesting their omnivorous nature (St-Hilaire et al. 2005; Gobbi et al. 2013; Nguyen et al. 2013; Tschirner & Simon 2015). However, a recent study has shown that individual colonies bred for longer than two years in different geographical locations and conditions expressed feeding plasticity (Zhou *et al.* 2013). This therefore shows a need to test the feeding abilities of all geographically dispersed populations of *H. illucens* to verify homogeneity in feeding abilities. This study will look to partly address this for one untested geographic location, South Africa.

Aside from the nutritional composition of the larval feed, another important variable that has been shown to significantly influence larval survivability, development rate and maximum size is feeding rate (mg of feed per larva per day) or provisioning ration (when larvae are fed once for the duration of experimentation). Diener *et al.* (2009) showed that *H. illucens* larvae feed optimally, in terms of waste reduction and biomass gain, when given 100 mg/larva/day and individuals are largest (and possibly more fertile (Tomberlin & Sheppard 2002) when given 200 mg/larva/day. However, the

resolution between 100 and 200 mg/larva/day is low and could therefore use further testing to assist when scaling up mass rearing of the larvae.

The aim of this study is to further understand how provisioning rations may affect feeding and developmental efficiencies (waste reduction, bioconversion, survival, prepupation) and dynamics (feed accessibility and provisioning ration changes) of the South African strain of *H. illucens*, under applied conditions. This will allow for better management and production for industrial mass rearing for companies using this strain of *H. illucens*. We hypothesise that with increasing provisioning rations, *H. illucens* larvae (1) will grow larger, (2) with better feeding and developmental efficiencies and thus (3) can access feed more easily over time and (4) larval nutritional quality will increase with increasing provisioning rations in terms of protein.

3.3 Methods and materials

3.3.1 Source of larvae

Methods undertaken to obtain larvae were consistent with those of Chapter 2.

3.3.2 Experimental design

Sixty clear cylindrical containers (2.8 Lt) were filled with 990 g of homogenised organic waste (sourced from the University of Cape Town; particle size: < 5 mm; pH: 4-5; depth: ±10 cm; moisture content: 75.12%). Six-day-old larvae were added in quantities of 1320, 880, 660, 528, 440 and 330 to equate to their six respective provisioning rations for 15 days. These were as follows: 50, 75, 100, 125, 150 and 200 mg/larva/day respectively (i.e. ten replicates of each; power (fixed effects) ≥ 0.9). Larvae fed for 15 days as suggested as the optimal duration of feeding for largest larvae was set as the experimental duration (Tomberlin et al. 2009; Gobbi 2012). A 500 g sample of the feed was sent for proximate analysis using standard AOAC methods (*Appendix 2*)(Quantum Analytical Services, Malmesbury, South Africa), and initial moisture contents were measured for larvae and feed using ten samples of feed and larvae each (*Appendix 2*). Larvae were added to the surface of the feed at the start of experiment, and the containers were sealed using Saffron cloth (Demtex, South Africa) and elastic to allow airflow. Experimental containers were then placed in 1 Lt tubs (to catch potential escaping larvae) 1 m from the floor in a controlled environmental chamber (26.98±0.6°C, 61.79±4.92% RH). All containers were placed in a Latin square design.

The following variables were recorded in all containers every two days from the second day of experimentation: feed and larval aggregation temperatures, feed depth (to calculate feed area accessed) and maximum depth of larval aggregation (taken from the bottom of the container as 0 cm).

A pencil thermometer and measuring tape were used to take recordings. All ambient temperature and relative humidity readings were taken using an iMonnit Humidity and Temperature Sensor (Monnit Corp, Utah, USA).

After 15 days, containers were weighed, 4-5 random sub-samples of feed residue were mixed and measured for final moisture contents of the feed (*Appendix 2*). The remaining residue was then washed away and larvae were collected in a sieve during washing, and dried on tissue paper. Ten fifth instar larvae from each replicate were then weighed individually on a Kern ABJ 320-4 Electronic Balance (decimal accuracy: 0.0001g) to obtain final instar mass. All larvae and prepupae were then counted and weighed from each replicate. Larvae moisture contents were taken and treatment replicates were combined to make 500 g samples which were towel dried and frozen to euthanise the larvae until sent for proximate analysis using standard AOAC methods (see *Appendix 2*) with Quantum Analytical Services (Malmesbury, South Africa).

3.3.3 Statistical analyses

All factors were analysed for conformity to the assumptions of normality of residuals and homogeneity of variances. Kruskal-Wallis tests with Tukey's HSD post-hoc tests were run to analyse waste reduction (%), bioconversions (%), biomass gained (%), survival (%), no. of prepupae (%), change in provisioning ration (%) and optimal bioconversion deficit (%). All variables investigated were non-parametric, due to a lack of homogeneity of variance ($p > 0.05$), except for the number of prepupae and dry biomass gained which were analysed as parametric.

Fifth instar mass variation was analysed with General Linear Model test using Statistica (StatSoft Inc. 2008). Feed accessed and feed depth lost was also analysed using a GLM as well. The correlation between provisioning ration changes and feeding efficiencies was also analysed using Pearson's correlation coefficient to assess relationships between the variables.

3.4 Results

3.4.1 Proximate analyses

Proximate analyses of the food and larvae are shown in Table 3.1. Crude protein (19.35-29.24%) of the larvae decreased with increasing provisioning ration while feed moisture (55.19-65.32%) generally decreased from 75 to 200 mg/larva/day but was lowest at 125 mg/larva/day, Crude fat (22.98-26.56%), ash content (3.37-5.16%) and crude fibre (3.81-6.12%) followed a similar trend to moisture content but with its' highest value being at 125 mg/larva/day.(Table 3.1).

3.4.2 Behavioural traits

The main variables analysed in this study are summarised and show that significance is seen for all variables analysed except maximum larval depth which is the only phenomenon in the analyses (Table 3.2).

Larvae took seven days to access > 90% of the feed in all treatments and after 15 days of feeding they could reduce the depth of the feed by ca. 30% across all treatments. The feed accessed was not significant between treatments but the feed depth lost was significant (GLM: $F = 1.48$, d.f. = 35, $p < 0.049$). Larvae generally increased their immediate environmental temperatures by as much as 4.8 ± 0.9°C across treatments with significant differences between 150 and 50 or 100 mg/larva/day, at 3.17 ± 0.88°C, 3.19 ± 1.1°C and 3.37 ± 1.06°C respectively. These temperatures were at their highest one day into feeding and decreased over time.

3.4.3 Feeding efficiencies

Waste reduction showed significant differences between treatments (Kruskal-Wallis test: $H_{(5, N = 60)} = 34.88328$, $p << 0.001$) and averages ranged between 25.7% and 35.4% (Dry Matter - DM) for 200 and 125 mg/larva/day treatments, respectively (Figure 3.13).

Bioconversion and efficiency of conversion of digested feed (ECD) values also show significant differences between treatments ranging between 6.3% (50 mg/larva/day) and 14.9% (125 mg/larva/day) (DM). For bioconversion (Kruskal-Wallis test: $H_{(5, N = 60)} = 44.59541$, $p << 0.001$) and between 18.4% (50 mg/larva/day) and 42.2% (125 mg/larva/day) for ECD values (Kruskal-Wallis test: $H_{(5, N = 60)} = 50.24984$, $p << 0.001$) (Figure 3.14).

There was significance between treatments (Kruskal-Wallis test: $H_{(5, N = 60)} = 48.99148$, $p << 0.001$) for larval survivability and was above 88.8% for treatments including and above 125 mg/larva/day (Figure 3.15a). Similarly, there was a significant difference between treatments for percentage prepupation (GLM: $F = 83.76$, d.f. = 5, $p << 0.001$) with 200 mg/larva/day being significantly higher than all other treatments (Figure 3.15b).

Provisioning ration changed significantly for treatments from 100 mg/larva/day and almost negligibly above that (Kruskal-Wallis test: $H_{(5, N = 60)} = 42.58808$, $p << 0.001$) (Figure 3.16). Provisioning ration change were also significantly correlated with average larval size ($r = - 0.76$).

Provisioning rations between 125-200 mg/larva/day expressed significantly higher optimal bioconversion deficits than the lower rations (Kruskal-Wallis test: $H_{(5, N = 60)} = 49.67$, $p << 0.001$) (Figure 3.17). The 125 and 200 mg/larva/day treatments were closest to maximum compared to other rates (Figure 3.17).

Individual final instar mass increased with increasing provisioning rations and differed significantly between all variables except between 50 and 75 mg/larva/day and 100-150 mg/larva/day (Figure 3.18a). Biomass gained showed a significant increase from 50-125 mg/larva/day treatments (GLM: $F = 161.47$, d.f. = 5, $p << 0.001$) up to 125 mg/larva/day treatments but there was no significance seen between 125-200 mg/larva/day as the values were similar (Figure 3.18b).

3.5 Discussion

3.5.1 Proximate composition of larvae

Proximate analyses of the diet was not significantly different from that of Chapter 2 and had the same sort of major concerns with the crude fat analysis method and NFE.Larvae showed significant increases in fat and decreases in protein when stressed with higher levels of competition. Many studies on other insects have shown this when larvae are put under stress via larval density or feed restriction, that may induce compensatory feeding for more fat-depositing nutrients to help alleviate the extra stress on the larvae (Green et al. 2003; Raubenheimer 2003; Ojeda-Avila et al. 2003; Hooper et al. 2003; Simpson et al. 2004). Considering the difference or lack of explanation as to how larvae were fed in many other studies, we cannot draw direct comparisons as we do not know the exact larval provisioning rations as well as due to some discrepancies in our own methods (i.e. crude fat analyses). What is interesting from these values, however, is that larvae did show changes in their composition du to the changes in density in this paper showing that intraspecific competition does have significant effects on *H. illucens.*

3.5.2 Feed accessed and aggregation heat

Very little is known about feed accessibility for *H. illucens* but the times taken to access the full depth (i.e. 10 cm) of the food coincided with Chapter 2:.

With respect to temperature changes, this is the first known record of larval aggregation temperatures for *H. illucens*. It is recommended to use temperature difference between ambient and aggregation as the most useful for industry due to varying environmental temperatures which will change the aggregation temperature observed due to fluctuating ambient temperatures (Reim et al. 2006; Slone & Gruner 2007). It is surprising that there is no significant increase in temperature with decreasing provisioning ration (or increasing larval density) as is suggested by Slone & Gruner (2007). This may be because temperature difference from ambient was not used in previous literature.

The aggregation temperatures are highest at the start of the study and decrease until the end of the study. This may show metabolic activity changes for *H. illucens* and/or the bacteria in the feed for the first time in literature (Mitchell-Foster et al. 2012; Verberk et al. 2015).

3.5.3 Feeding efficiencies

Waste reduction values showed that 200 mg/larva/day was significantly lower to the other treatments (Fig. 1) while all other treatments reduce waste at similar levels. This might indicate that *H. illucens* have a carrying capacity for larval densities (and thus waste reduction) in a set volume of feed, and high mortalities in the denser treatments (50-75 mg/larva/day) support this (Figure 3.15a). In terms of literature, this paper has shown values which are lower than others recorded but this may be due to varying feeding durations and feed compositions, so no rigorous comparisons can be made (Diener et al. 2009; Zhou et al. 2013; Gabler 2014).

It may be necessary to evaluate how survival of larvae changes over experimental time in the form of waste reduction indices or survival curves as changing survival rates would inevitably affect the larval feeding abilities such as waste reduction. This is poorly understood for set provisioning rations but there is minimal research for daily feeding regimens (Slansky & Scriber 1982; Diener et al. 2009). For the sake of this study, waste reduction was chosen because it is believed that when mass rearing insects you would want to achieve the highest biomass in the shortest known time, therefore optimal temperatures and larval development times would need to be fixed in this case, as per recommendations from Tomberlin et al. (2009) to assess the effect of provisioning rations on feeding efficiencies.

Bioconversion and ECD show high values for treatments above 125 mg/larva/day but in terms of bioconversion it reverses its trend after this treatment and decreases significantly (Figure 3.14a) whereas in ECD values it reduces but is similar in both higher treatments (Figure 3.14b). Peculiarly, the ECD values contrast with the study of Diener et al. (2009) which shows an opposite trend; this may be due to the higher number of individuals used in this trial and differences in experimental design and feed. However, where applicable, it seems ECD values were comparably higher than those given in Diener et al. (2009).

Optimal bioconversion deficit (OBD) value is a completely new concept; along with change in provisioning ration and it is believed they could have value for industry in terms of evaluating and managing feeding efficiencies of insect cultures. Optimal bioconversion deficit gives a good indication of how close a population is to the theoretical maximum (based on averages for the variable inputs) thus allowing assessment of whether any or all variables are causing a deficit of the potential of the larvae to feed in that set environment. In this study, it is seen that larval density has a significant effect on OBD at the 50-100 mg/larva/day treatments and this may be due to higher levels of stress

due to increased competition at these provisioning rations. This was not the case from 125-200 mg/larva/day but 125 mg/larva/day showed very little variability in the data and suggests that larvae can feed with great stability at these provisioning rations across replicates, but are not adversely effected if the larvae are given even higher provisioning rations (Figure 3.17). For more stringent application, the formulae and consistency of the data produced from it should be investigated further. Similarly, the provisioning ration change does something very similar but may show how mortality of larvae may affect larval mass and feeding efficiencies too. Although, it is necessary to validate its scientific usefulness and a mortality curve would make the equation more useful.

3.5.4 Survival and prepupae

Survival rates for 125-200 mg/larva/day are higher when compared with other literature (regardless of experimental design differences) (Tomberlin et al. 2002, 2009; Myers et al. 2008; Yu et al. 2011), but all treatments below this show much lower survival rates (Fig. 3.1). This can most likely be attributed to intraspecific competition (Reim et al. 2006; Mitchell-Foster et al. 2012) and feed (Ojeda-Avila et al. 2003). Interestingly the optimum of 100 mg/larva/day; as suggested by Diener *et al.* (2009) had a lower survival rate than the 125 mg/larva/day optimum from this paper. This may show that when considering biomass and feeding efficiencies, it is imperative to consider survival rates of larvae, as this will have cascading effects. Additionally, it is necessary to understand where this mortality happens during experimentation to know how much time larvae could feed for at specific larval densities as previously mentioned.

In a *H. illucens* mass rearing facility it is important to understand all factors affecting the duration to 5th ecdysis, i.e. turning from L5 larvae to prepupae, as this will affect the quality of any larval-derived product (Park et al. 2013). This study shows that within 21 days (from hatching) there is only a significant proportion of prepupae in the population at 200 mg/larva/day. Therefore, to limit pre-pupae and maximize quality of a larval-derived product (i.e. largest, most nutritious individuals; Gobbi 2012) 125 mg/larva/day or lower should be given or if a quicker developing population is wanted, 200 mg/larva/day is most efficient.

Larvae were larger than most previously known from other studies (Tomberlin et al. 2002, 2009; Myers et al. 2008; Diener et al. 2009; Bonso 2013; Zhou et al. 2013; Gobbi et al. 2013; Nguyen et al. 2013; Banks et al. 2014; Oonincx et al. 2015a, 2015b). There is a need to investigate why this is the case and possibly the effect of larger larval size on adult fecundities (i.e. effect on the next generation). Interestingly, biomass gained was non-significant from 125-200 mg/larva/day and therefore it is possible that larvae can only bioconvert to a certain capacity at present conditions at higher treatments.

In conclusion, larvae grow larger with increasing provisioning ration but feeding efficiencies and select life history traits (survival and development) either become, or are, different above 125 mg/larva/day. Additionally, larvae were more nutritious with respect to protein with increasing provisioning ration, and feed accessibility is not affected by change in larval densities. Therefore, larvae from the South African population seem to differ in feeding efficiencies from other populations, supporting geographic plasticity (Zhou et al. 2013), but this needs further research on specific feeds from all strains to confirm this. Finally, to achieve the largest larvae with the best feeding efficiencies and survival in the quickest time; 125 mg/larva/day as a provisioning ration at temperatures around 27°C is optimal at present larval densities and provisioning rations. Like previous studies, here we suggest that this be tested when scaling up the population sizes of *H. illucens* to confirm if changing the size of the population (and their feeding environment) affects life history traits (Diener 2010; Diener et al. 2011b; Bonso 2013; Devic et al. 2014). Additionally, it may be necessary to assess the economic viability of changing the size of the population we rear *H. illucens* larvae at to understand what size of containers (and larval densities) industry could be using in the future.

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3.7 Tables

Table 3.1: Nutritional composition results for the homogenised feed fed to *Hermetia illucens* larvae and for the larvae at the end of the experimental phase. All percentages are based on 100% dry matter.

RATION (MG/LARVA/DAY	*MOISTURE CONTENT (%)*	*CRUDE PROTEIN (%)*	*CRUDE FAT (%)*	*CARBOHYDRATES (CHOS) (%)*	*ASH CONTENT (%)*	*CRUDE FIBRE (%)*
HOMOGENISED FEED	**77.28**	8.11	6.85	11.54	2.45	0.45
50	65.32	19.35	22.98	1.53	4.56	4.67
75	63.75	20.85	25.07	2.64	4.49	3.83
100	61.46	22.80	25.69	5.81	4.60	3.81
125	55.19	27.60	27.03	15.27	5.16	6.12
150	55.30	29.04	26.56	15.15	4.97	5.10
200	58.38	29.24	23.12	10.19	3.37	5.36

Table 3.2: Statistical significance summary for all main variables analysed between provisioning rations. Sig. = Significance.

VARIABLE	STATISTIC	N	D.F.	F/H	SIGNIFICANCE (P>0.5)
Survival	K-W ANOVA	10	5	48.99	Sig.
Prepupae	GLM	10	5	83.76	Sig.
Bioconversion	K-W ANOVA	10	5	44.60	Sig.
Waste Reduction	K-W ANOVA	10	5	34.88	Sig.
OBD	K-W ANOVA	10	5	49.67	Sig.
PRC	GLM	10	5	87.71	Sig.
Max Diet Depth	GLM	10	40	1	n.s
Diet lost	GLM	10	35	1.48	Sig.
Mass gained	GLM	10	5	161.47	Sig.
Larval Mass	RMANOVA	10	50	208.60	Sig.
ECD	K-W ANOVA	10	5	50.25	Sig.

3.8 Figures

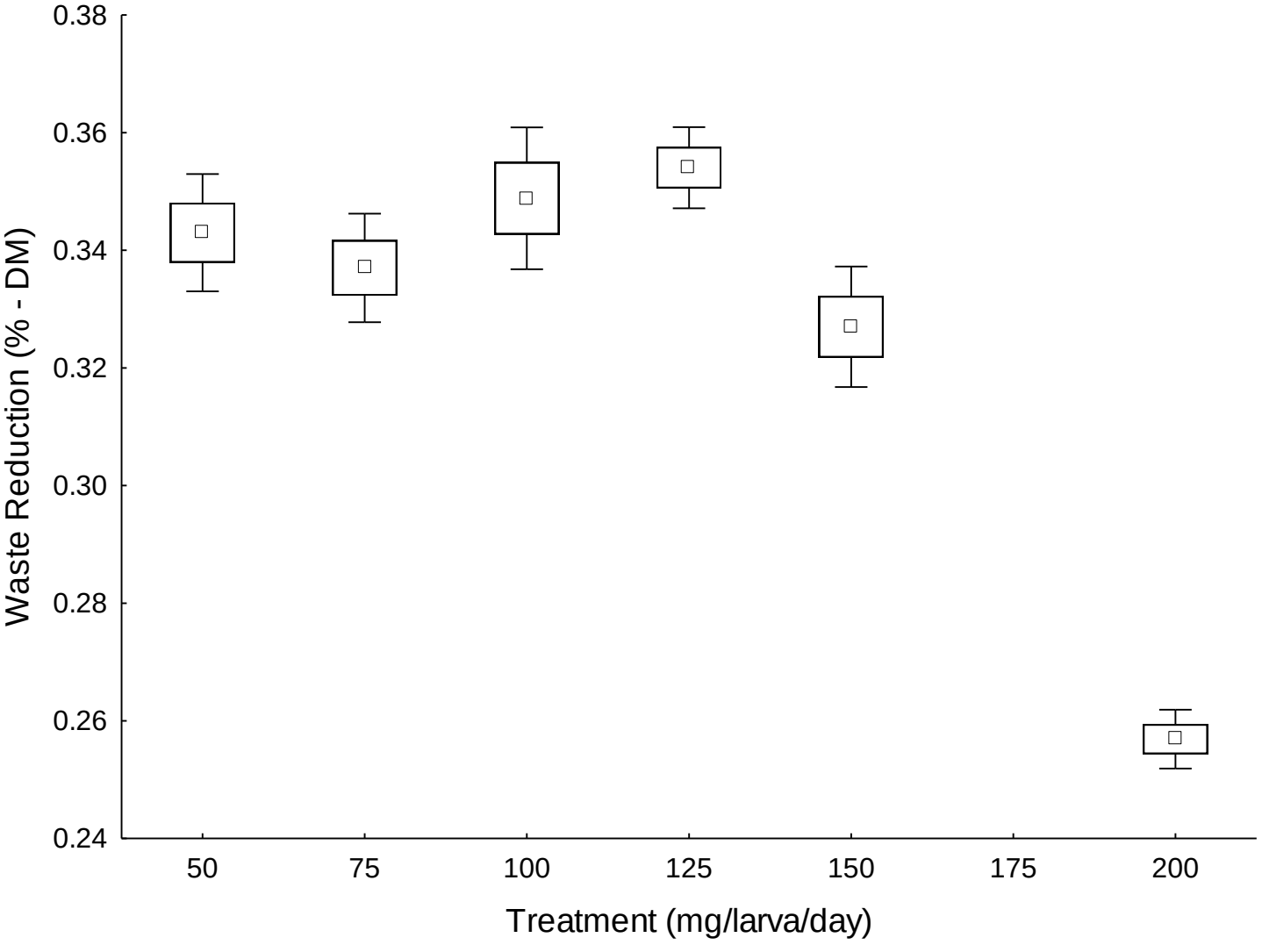

Figure 3.13: Waste Reduction (% - DM) for the black soldier fly, *Hermetia illucens*, at six different provisioning rations (mg/larva/day). Boxes denote mean and standard error (SE); whiskers denote 1.96*SE.

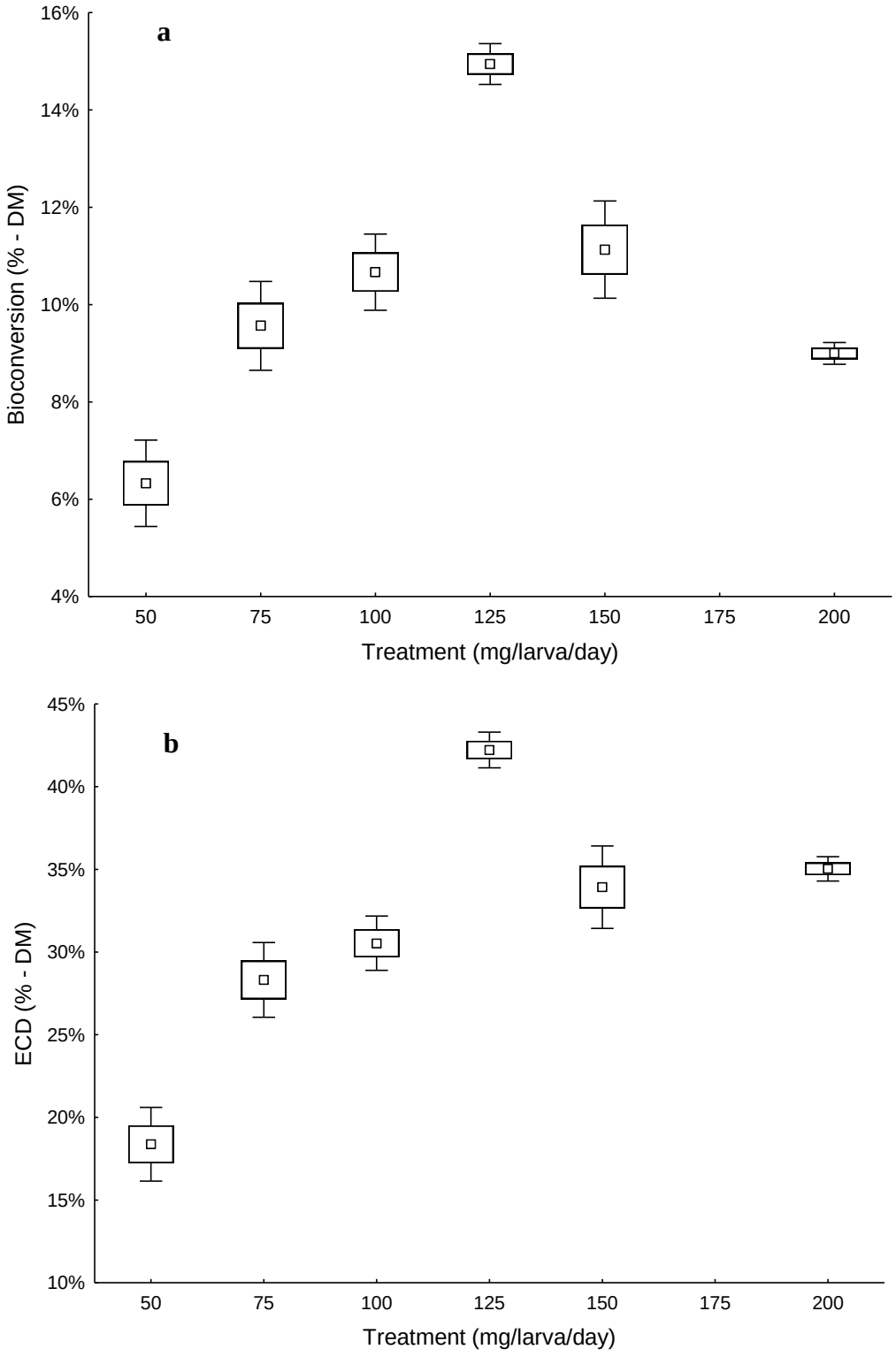

Figure 3.14: a) Bioconversion rates (% - DM) and b) efficiency of conversion of digested-feed (ECD) (% - DM) for the black soldier fly, *Hermetia illucens*, at six different provisioning rations (mg/larva/day). Boxes denote mean and standard error (SE); whiskers denote 1.96*SE.

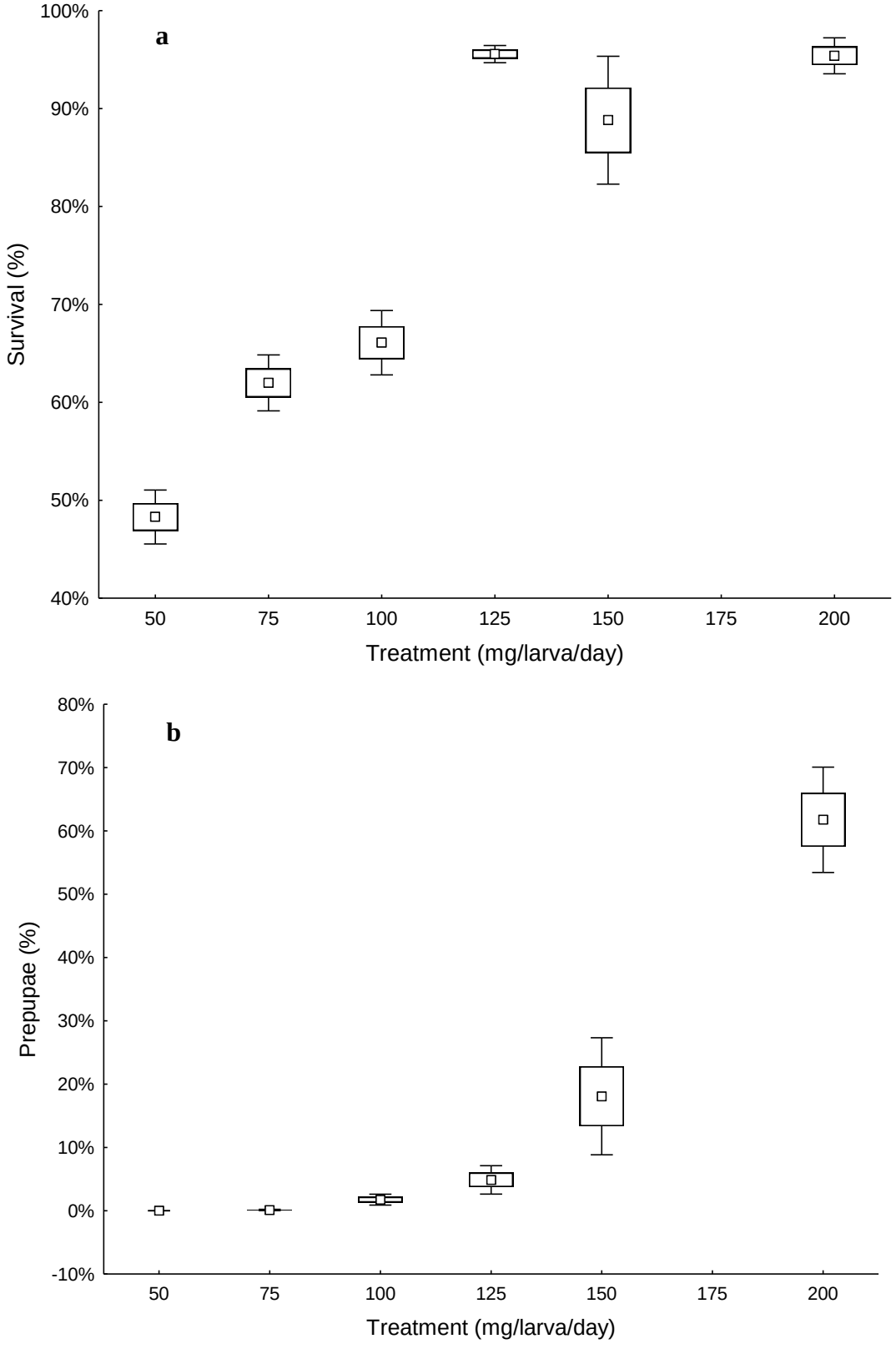

Figure 3.15: a) Survival (%) and b) prepupae (%) for the black soldier fly, *Hermetia illucens*, at six different provisioning rations (mg/larva/day). Boxes denote mean and standard error (SE); error bars denote 1.96*SE.

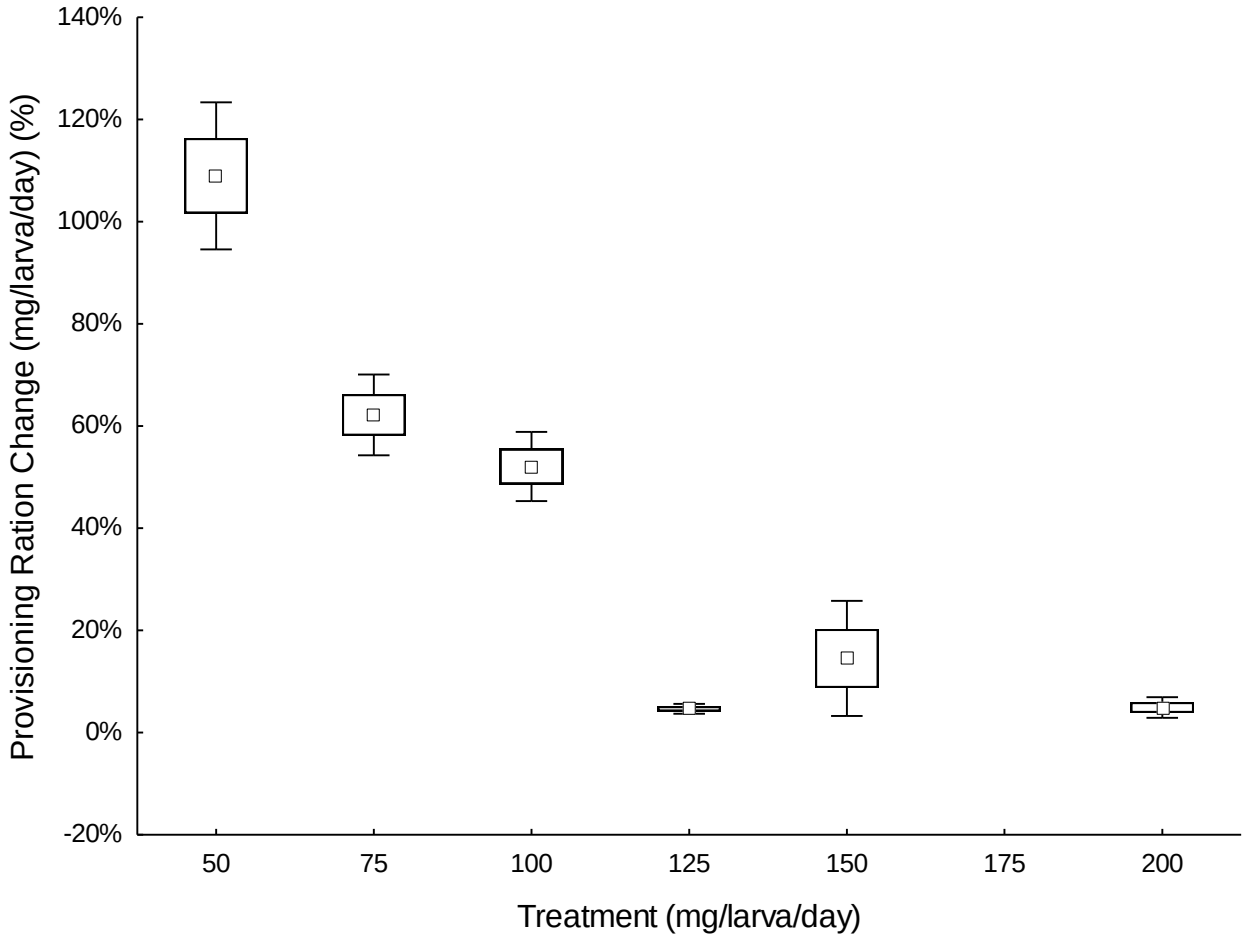

Figure 3.16: Provisioning ration change (mg/larva/day) for the black soldier fly, *Hermetia illucens*, at six different provisioning rations (mg/larva/day). Boxes denote mean and standard error (SE); error bars denote 1.96*SE. The change in provisioning rations takes both the food and number of larvae into account at the beginning and end of the experiment

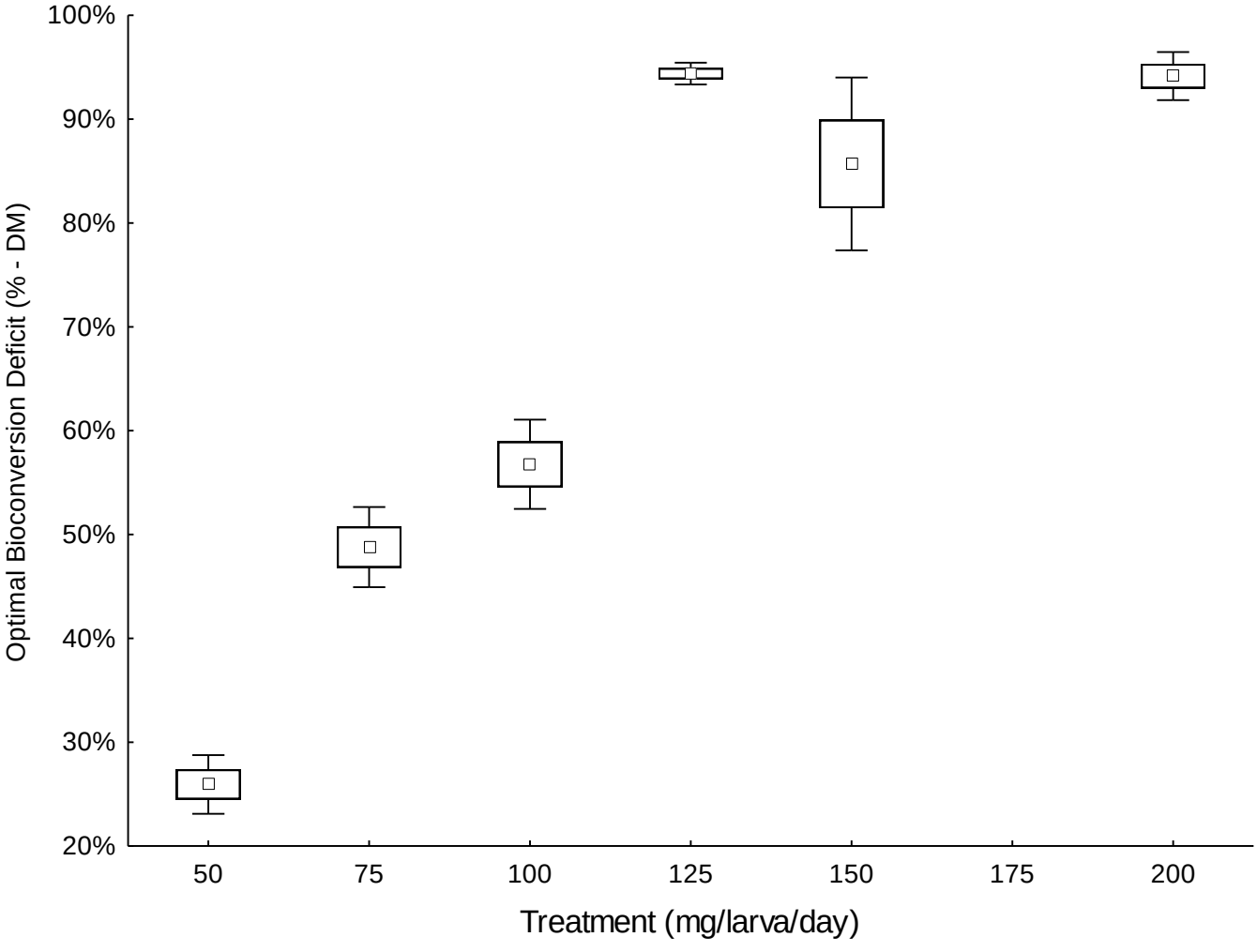

Figure 3.17: Optimal bioconversion rate offset (% - DM) for the black soldier fly, *Hermetia illucens*, at six different provisioning rations (mg/larva/day). Boxes denote mean and standard error (SE); error bars denote 1.96*SE.

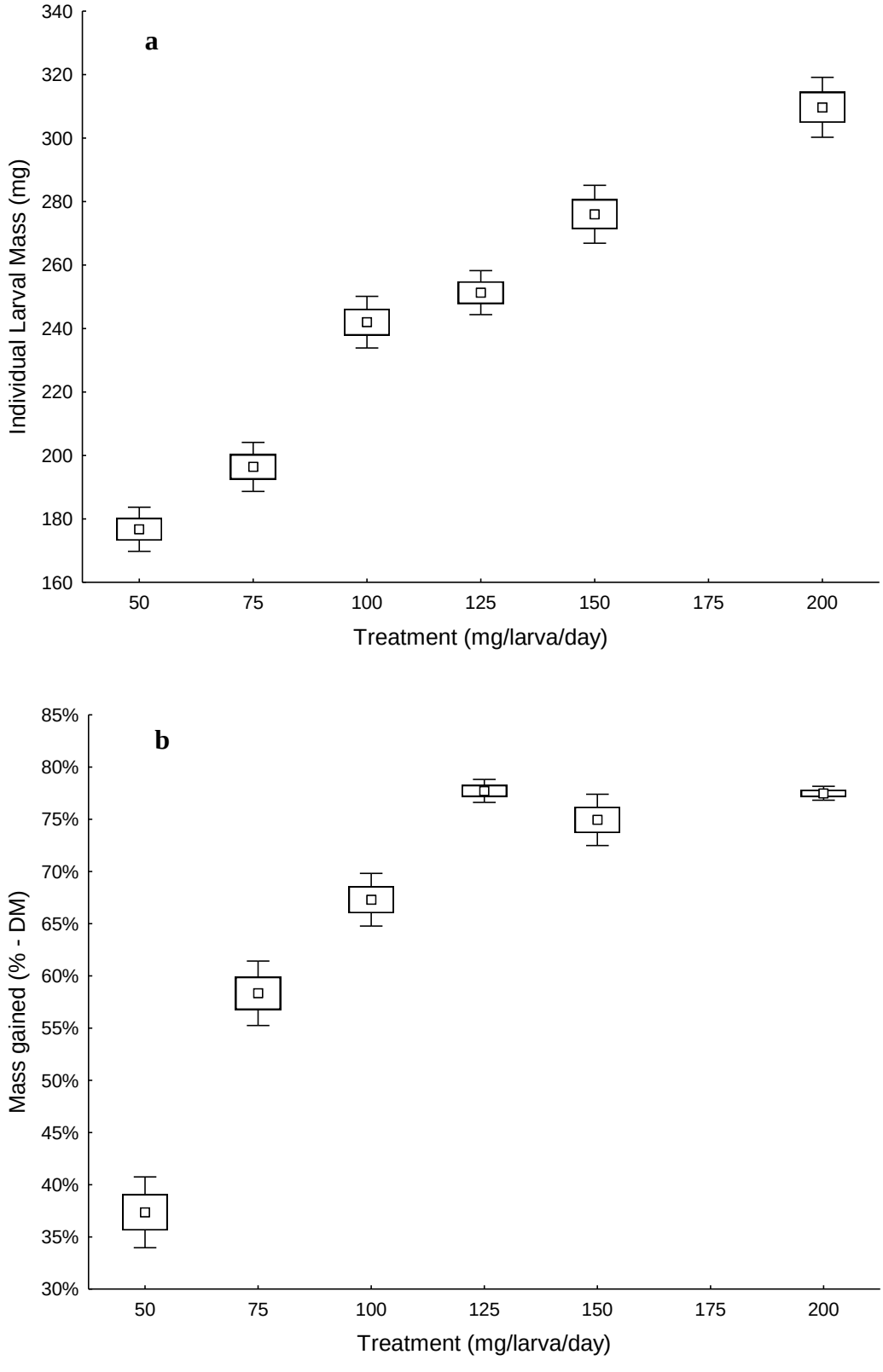

Figure 3.18:a) Individual final instar mass (mg) and b) mass gained (% - DM) for the black soldier fly, *Hermetia illucens*, at six different provisioning rations (mg/larva/day). Boxes denote mean and standard error (SE); error bars denote 1.96*SE.

CHAPTER 4:

POPULATION SIZE

4. The feeding efficiencies and life history traits of the black soldier fly larvae, *Hermetia illucens* (L., 1758) (Diptera: Stratiomyidae) at five different population sizes

4.1 Abstract

Contemporary research has focussed on the mass rearing and up-scaling of this fly for feed production for livestock (i.e. poultry, swine and aquaculture) around the world due to *H. illucens*' characteristics. However, the effect of population size has never been investigated. It is believed that changing the number of individuals kept per container may have effects on values such as bioconversion, body mass and waste reduction of these flies, which could affect the growing industry. This paper uses five treatments of population size in orders of magnitude between 5 and 50 000 individuals to test how this variable affects feeding efficiencies. Larval body mass reduces as population size increases but variation decreases above 500 larvae treatments. Additionally, significant differences were noted between treatments in waste reduction, bioconversion, and crawl-off biomass. Bioconversion showed a promising linear increase with treatment but it is recommended that harvesting methods should be evaluated for future research. Other factors that might affect the results, such as crawl-off, harvesting methods, feed depth lost, and larval moisture content are discussed. Future recommendations to improve harvesting techniques and use of the largest possible population sizes was recommended if harvesting techniques can be better quantified in future research.

Keywords: Integrated biosystems, waste management, mass rearing, population sizes, bioconversion rates, ecology, organic bioconversion.

4.2 Introduction

Sheppard et al. (2002) have shown that it is possible to rear *H. illucens* in captivity for multiple generations in the laboratory. But there is growing commercial interest in rearing *H. illucens* on an industrial scale and these experiments do not represent conditions for *H. illucens* under mass rearing (Devic et al. 2014). Recent attempts have been made in Ghana and uncovered some interesting constraints in the rearing process of such large numbers of larvae, particularly that their methods were time consuming and the larval production rates were inconsistent (Devic et al. 2014). One important factor in scaling up is identifying an optimal larval population size per container, as this affects material costs and possibly production efficiencies. This is challenging as population dynamics with such increases could cause stresses on the population which could result in instability or collapse (Stiling 1988; Jarošik & Dixon 1999; Green et al. 2003). *Hermetia illucens* larval density has already been shown to affect survival, development rates, bioconversion, and individual size and nutrient composition (see Chapter 3:) but no work has looked at how the change in larval population size may affect these factors, even when feeding density is kept consistent.

Thus, to better understand the effect of population size on the life history of *H. illucens*; this study looks at how larval feeding efficiencies are affected in different population sizes. It is hypothesised that: 1) increasing population size will cause increases in bioconversion and waste reduction ability of larvae to a carrying capacity and 2) final fifth instar larval mass will be larger at smaller population sizes above a certain population size but will be smaller in size due to competition stress past this threshold.

4.3 Methods and materials

All work was carried out in controlled environment rooms at the Research and Development Department at AgriProtein Technologies in Cape Town, South Africa (34°0'8.2" S; 18°36'16.4" E).

Two sub-populations of adult *H. illucens* were reared in two suspended 2.53 m^3 nylon-mesh cages since April 2015 under controlled, near-optimal conditions (temperature: mean = 30.08, SD = 2°C (Tomberlin et al. 2009); relative humidity (RH): mean = 72.88, SD = 10% (Holmes et al. 2012)). This setup was like the medium cage setup in Gobbi (2012), Adults were supplied with water *ad libitum* and artificial light conditions to ensure optimal mating and oviposition conditions (Sheppard et al. 2002; Zhang et al. 2010). The populations were obtained from a colony started at AgriProtein Technologies in February 2012 (Elsenberg, Western Cape, South Africa) incorporating both locally-obtained wild and captive populations. A covered oviposition attractant (kitchen waste < 2 days old) exposed in the cages with proprietary egg grids, suspended no more than 2 cm above the attractant, was presented as an oviposition site (Sheppard et al. 2002). Adults were given ca. 24 h to oviposit after introduction of the grids, which were then removed from the cages.

Hermetia illucens eggs from several grids were placed inside a proprietary collection apparatus in an environmentally controlled room at ca. 27.5°C and 90% relative humidity. Eggs were thereafter given 96-105 h (Sheppard et al. 2002) to hatch and were collected in shallow 4 Lt containers as neonate larvae.

4.3.1 Experimental design

Neonates were placed on food in containers at five different population sizes in orders of magnitude from 5 to 5.10^4. Larvae for the 5 and 50 larvae treatments were counted out and

added to their containers while all other treatments were added by weight, using a calculated 25.3 µg weight per individual (Badenhorst et al., unpublished data). This was done as the two smallest treatments showed high variation when added by weight in a pilot study.

A mixed and homogenised kitchen and pet food waste was provided as feed for the larvae; they were fed twice during the experiment; at day zero and seven at an average of 100 mg/larvae/day (i.e. 125 mg/larva/day for the 15-day trial period) provisioning rations for 21 days. Additionally, samples of 500 g of feed were sent for proximate analysis by Quantum Analytical Services (Malmesbury, SA). The depths and pH of the food (pH = 5) were taken and an iMonnit Temperature and Humidity Sensor (Monnit Corp, Utah, US) was used to monitor ambient temperature and humidity in the experimental room. Containers were chosen for each treatment with increasing volumes to maintain a population density of ca. 2.12 g/larvae. During the first six days, larvae were left to grow undisturbed to reduce mortality (Tomberlin et al. 2002, 2009).

Due to variable access to standardised containers per treatment and some containers being proprietary in nature; surface area, volume and depth were all measured at the beginning of the experiment for each container.

Each treatment was replicated 12 times in an environmentally controlled facility (26-30°C, 50-80% RH). Containers were placed inside collection trays for any crawl-off larvae to be collected and quantified during the experimentation, and were removed from the experiment as this simulates usual crawl-off collection in currently designed systems (Newton et al. 2004; Diener 2009; Kroes 2012). After the 6th day, containers were checked every 2-3 days for size and consistency of feed and any crawl-off was weighed from each of the treatments and removed. The crawl-off biomass was split equally between all replicates within each catchment system at each interval.

On the 21st day, containers (and all their contents) were weighed and all residue and larvae were separated using recycled water and a five millimetre sieve and shade cloth as a larval catchment system. Larvae were then cleaned further and excess water was removed and larvae weighed. Residue was calculated via deduction of the container weight and larval biomass. All feed and larvae were also sampled at the start and end of experimentation for moisture content and calculated using 5 g samples dried for 24 h at 80°C, with weight lost during this period being the moisture content of the sample.

4.3.2 Statistical analyses

Power analyses and basic General Linear Models (GLM) assumption checks (distribution fitting, p-plots and a Shapiro-Wilks W t-Test) were performed on all variables. Power analysis showed that the twelve replicates done for this study resulted in a power of 0.99, allowing for strong inference of the data to be made to any population under similar conditions.

General Linear Models (GLMs) was used to analyse larval moisture content, waste reduction and feed depth lost (i.e. the amount of feed depth reduced per day as a percentage of its total depth), while final instar larval mass, crawl-off biomass and bioconversion (% - DM) were analysed by Kruskal-Wallis ANOVAs. Change in moisture content of the larvae was also analysed and area, volumes and surface areas of the feeds were compared between treatments and correlations were run between these and all other variables to see if spatial differences may have had any impact on the experiments.

4.4 Results

4.4.1 Feed composition

Feed showed a nutritional composition (dry matter basis) of 2.45% ash, 5.29% crude fat, 0.45% crude fibre, 11.54% CHOs (i.e. Starches and sugars), 8.11% protein and 78.28% moisture.

Larval moisture content change increased from the initial moisture at the start of the experiment significantly from above the 500 larvae treatments (mean range = 10-12%) while less for the 5 and 50 treatments (mean = 6%; Kruskal-Wallis test: $H_{(4, N = 60)} = 35.51$, $p << 0.001$).

4.4.2 Feed depth lost and waste reduction

Feed depth lost over the course of the experiment showed significant differences between treatments (Kruskal-Wallis test: $H_{(4, N = 60)} = 47.89$, $p << 0.001$), particularly for 5 and 500 treatments, and all other treatments with the greatest change in depth being observed at the 5000 larvae treatment (mean = 61.8%) followed by 50 000 larvae, then 5, then 500 and finally 50 (mean = 19.0%) being lowest in feed depth lost.

Larvae could reduce waste significantly between most treatments (Kruskal-Wallis test: $H_{(4, N = 60)} = 41.87268$, $p << 0.001$), with the extremes being between 500 (mean = 55.0%) and 5000 (mean = 82.7%) (Figure 4.1).

4.4.3 Bioconversion and crawl-off

Bioconversions showed a positive linear trend as the population size grew (Figure 4.2). Significant differences were seen for all treatments (ANOVA: $F = 54.33$, d.f. = 4, $p << 0.001$) except between 5 and 50 larvae ($p = 0.94$), and 5000 and 500 larvae ($p = 0.76$).

Crawl-off biomass was most significant (ANOVA: $F = 24.82$, d.f. = 4, $p << 0.001$) in the lower treatments of 5 and 50 (16-19% Wet Mass (WM)) while being similar between 500-50 000 (2-3% WM). Significant differences were not seen between 5 and 50 larvae (Tukeys: $F = 24.82$, d.f. = 4, $p = 0.92$), 5000 and 50 larvae (Tukeys: $F = 24.82$, d.f. = 4, $p = 0.97$), 50 000 and 500 larvae (Tukeys: $F = 24.82$, d.f. = 4, $p = 0.998$) and finally between 50 000 and 5000 larvae (Tukeys: $F = 24.82$, d.f. = 4, $p = 0.995$). Additionally, crawl-off biomass and bioconversion were highly correlated ($p = -0.82$) where the increase in crawl-off was correlated with a reduced bioconversion.

4.4.4 Surface- area- to- volume ratios

Surface-area-to-volume ratio of the different containers used in each treatment showed an exponential trend that was significant from one another as expected (ANOVA: F = 20022624, d.f. = 4, p << 0.00001). Pearson's correlations however found no significant relationship (r > 0.7) between SA: Vol. and all feeding efficiency variables.

4.4.5 Larval mass

Fifth instar larval mass was shown to be significantly different (ANOVA: F = 17.899, d.f. = 4, p << 0.001) ranging from 65-86,4 mg (DM) but significance only occurred between 50 and all other treatments (Tukeys: F = 17.899, d.f. = 4, p << 0.0001) (Figure 4.3). There was a notable variability in the 5 larvae treatments compared to all other treatments where variability (SD = 30.31 (5 treatment) - 13.23 (50 000 treatment)) become less pronounced as treatments increased.

4.5 Discussion

4.5.1 Feed composition and larval moisture content

Larvae were fed feed that fell within known, tested ranges with other *H. illucens* studies for protein, lipids and moisture content (Nguyen et al. 2013). The values are also non-significant from Chapters 2 and 3 but contain the same discrepancies with crude fat and NFE analysis.

Hermetia illucens larvae seem to be extremely fragile as a population with high occurrences of mortality during their first two instars possibly due to their high surface-area-to-volume ratios and fragile body structures (Sheppard et al. 2002; Tomberlin et al. 2002). Results show that neonate larvae had a lower moisture content than fifth instar larvae. Due to the minute size of neonate larvae, they have a far higher surface-area-to-volume ratio compared to their fifth instar counterparts, which promotes moisture loss. This moisture loss most likely occurred

during the experimental setup where neonates were left exposed during feed preparation. This was less than 6 hours and therefore it is advisable that neonates not be held for longer than six hours off feed or a wet medium after hatching because this could potentially affect their successive life stages. Additionally, the smaller population sizes showed less moisture gained by the larvae than the other trials with more individuals in the population. This is poorly understood but it is hypothesised that it occurred due to less movement of the larvae, and thus less feeding (and therefore less moisture gained), and possibly lower aggregation heating effects on metabolism in the smaller populations than in those of the larger populations.

4.5.2 Feed depth lost and waste reduction

Waste reduction and feed depth lost showed very similar trends between treatments. However, waste reduction values (especially the larger treatments) was high compared to other literature at around 40-60% (Newton et al. 2004; Diener et al. 2009, 2011; Diener 2010; Zhou et al. 2013; Lalander et al. 2014, 2015). However, these higher values in the latter treatments are consistent with other parts of this thesis (see Chapter 2: and Chapter 3:). This contrast was possibly caused by the method of separating larvae from waste, which has been shown to be of concern (Devic et al. 2014; Maquart et al. 2016). Due to its novel and simple nature, the system was not able to separate larvae and all the feed (i.e. harvesting). However, harvesting efficiencies were equivalent between treatments, therefore it is believed that data between treatments are still comparable. Feed depth lost showed similar values to that shown in previous studies done at the 500 larvae treatment size (see Chapter 3:). It is advised that further research should establish a clean way of harvesting *H. illucens* and their feed medium or quantify more thoroughly the amount of waste removed with *H. illucens* using this harvesting technique.

4.5.3 Bioconversion and crawl-off

Crawl-off and bioconversion have very often been used to assess the performance of larvae for waste management (Newton et al. 2004; Diener 2010; Diener et al. 2011; Banks 2014). However, what has never been shown before is the significant correlation between these two variables. Crawl-off implies that larvae will be collected and thus stop feeding. This, therefore, ends their bioconversion potential depending on when crawl-off occurs. This means that not only is potential biomass gain lost but the provisioning ration of the larvae is changed (see Chapter 2:). Additionally, a lot of literature recommend collecting crawl-off larvae (Sheppard et al. 1994; Sheppard 2002; Newton et al. 2004, 2005; Diener et al. 2011; Banks 2014). While this seems to be useful at lower population sizes, no more than 5% of the population is collected within 21 days at optimal conditions. This shows a significant loss in biomass and therefore it is suggested that containers be designed so that larvae do not crawl-off or by feeding them back into the container if they do exit the feed in a cost-effective manner. Designs have already been created to assist in this and should be considered in future feeding container designs (Kroes 2012).

Bioconversion are comparable with values from other literature in the lower treatments but are often slightly higher in most cases, although many studies used a variety of specific feed mediums (10-14% in some cases (Diener 2010; Banks & Cameron 2012; The Biocycle 2012; Zheng et al. 2012; Bonso 2013; Banks 2014; Banks et al. 2014; Cheng & Lo 2015; Oonincx et al. 2015; Paz et al. 2015; Surendra et al. 2016) but in one study above 34% (Li et al. 2015)). Higher treatments showed much higher values than literature, this may have been caused by the methods of harvesting which may have influenced the values of bioconversion. However, the trends suggest that there is more biotic potential if the population is increased beyond 50 000 larvae per container. Some research has attempted this and seen good results, although

mostly observational (Newton et al. 2005). It is suggested that, due to the time-consuming nature of harvesting in the 5 000 and 50 000 larvae treatments, that population sizes larger than these not be investigated until more accurate harvesting methods have been devised as these harvesting errors may be amplified.

4.5.4 Larval mass

Individual final larval mass showed contrasting results to that of feeding efficiencies. This was unexpected because feeding efficiencies and larval mass have previously been closely correlated (Banks et al. 2014). Two reasons may explain this. First, this study looked at dry matter of the larvae over wet matter, unlike most other studies. This may mean that moisture content may be affected by these treatments and therefore dry matter analysis was better. Second, and more likely, the larvae in the 50 larvae treatments had early crawl-off that increased the remaining larval provisioning rations and therefore allowed them to grow bigger on average (see Chapter 3:; Diener et al. 2009). These factors should be considered in future investigations. Additionally, treatments above 500 larvae showed a possible carrying capacity for weight with reduced variability which may suggest that larvae, while being smaller, may be uniform in size. This may be advantageous when planning a system that requires stable biomass inputs to create specified product masses for supply and demand purposes.

In conclusion, larvae can reduce waste well across all treatments without significant negative effects on feeding efficiencies. However, better harvesting methods may be necessary to confirm the values given in this study. There is more biotic potential for bioconversion, since bioconversion increased with stabilising larval mass variation. However, by increasing population size past the highest treatment here, stress may start to influence larval size and other variables due to overcrowding (Hooper et al. 2003; Brent 2010; Mitchell-Foster et al. 2012). This requires future research as it may also lead to even greater bioconversions but a

better harvesting system is needed first. Future research should additionally look at how these larvae can accumulate nutrients across these treatments as this may be effected by change in population size.

4.6 References

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4.7 Figures

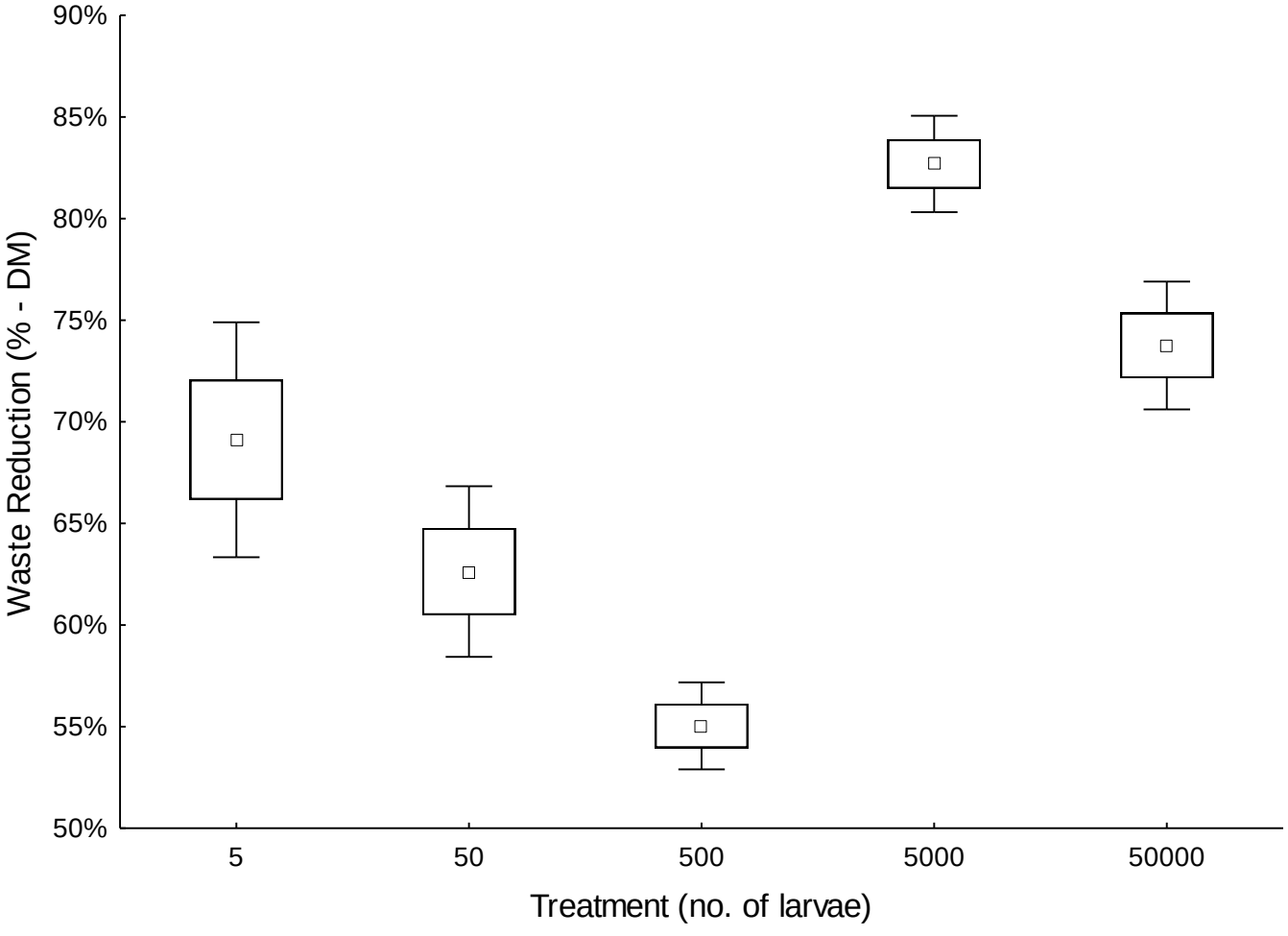

Figure 4.19: Waste reduction values (% - DM) for five different population sizes of black soldier fly larvae, *Hermetia illucens*. Boxes denotes standard errors and bars denote 1.96 SE.

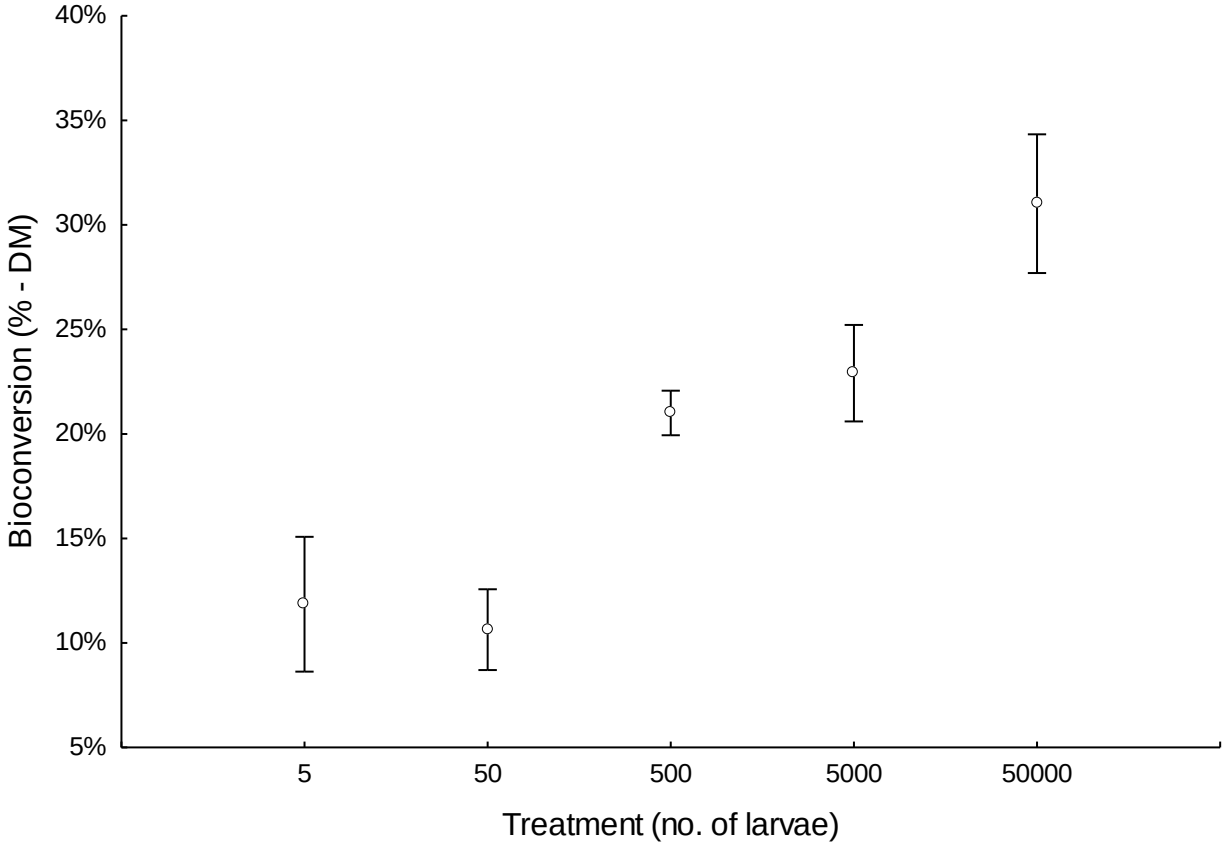

Figure 4.20: Bioconversion values (% - DM) for five different population sizes of black soldier fly larvae, *Hermetia illucens*. Error bars denote standard errors.

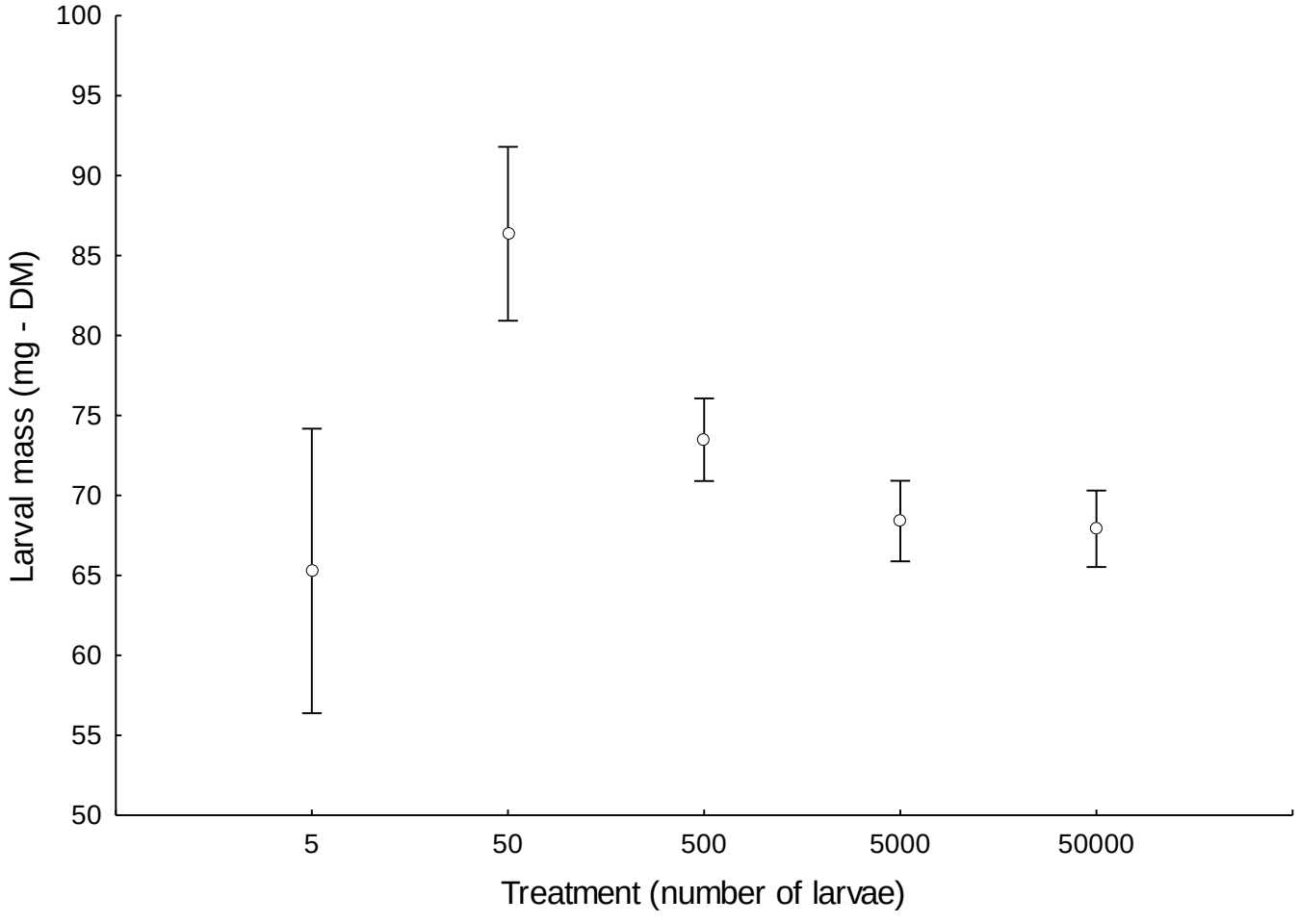

Figure 4.21: Individual fifth instar larval mass (mg - DM) for five different population sizes of black soldier fly larvae, *Hermetia illucens*. Error bars denote standard errors.

CHAPTER 5:

CONCLUSIONS AND FUTURE RECOMMENDATIONS

5. Conclusions and future recommendations

Hermetia illucens can be used in a variety of applications such as reducing landfill use, greenhouse gas emissions, environmental eutrophication/degradation, waste management and food security making it one of the most useful insects.

While the turn of the 21st century has seen significant growth in researching this species, much is lacking on how we can create stable conditions and production of these flies in mass rearing setups on industrial scales (Chapter 1:). This is essential considering the demand for food security and the ever-increasing amount of waste that society produces. Not only will this increase food security directly but may also do so indirectly by alleviating some of the demand for fish or monoculture plants such as soya bean and rapeseed.

This thesis has taken a few steps towards better understanding how larval feeding dynamics and feeding environment play a pivotal role in feeding efficiencies. Additionally, insights were given into how larval feed densities and feeding efficiencies change over time and their impacts on life history. Larval aggregation heat was also recorded and the individual and population effects were seen at multiple levels using applied settings.

In Chapter 2:, I show the effect of feed depth and particle size over two different generations.

What was most evident in this study was that there will always be negative impacts on both life history and feeding efficiencies when diet depths increase, most likely due to the change in feed accessibility. But, feeding depths at 5 and 10 cm produce similar rates of access with slight differences in survival and feeding efficiencies between the two treatments. Five centimetre treatments did show the most prepupae during this experiment and suggests that larvae will develop faster over the same amount of time when feeding at 5 cm. This shows that larvae cannot feed at depths greater than 10 cm without negative impacts on their feeding abilities. What may be of interest is seeing how this changes over several generations in an industrial setting and should be the topic of future research.

Additionally, minor increases in larval size and feeding efficiencies were noticed when the feed particle size was decreased. This can also be attributed to better access of the diet. It could be important to better understand how particle size effects the larval feeding efficiencies as, particle sizes were only reduced to a maximum size (i.e. the particles could range below this size variably), while there may be an optimal for consumption and environmental conditions within this variation.

Two new measures of feeding efficiencies were also introduced and correlate with measures such as waste reduction and bioconversion, while providing an estimate of larval feeding efficiencies. However, it must be acknowledged that these formulae will only be as strong as the data used to build them and therefore accuracy of data collection and calculations is crucial.

This study also showed that larval masses can heat their immediate environment by no more than 6°C when kept at optimal provisioning rations when reared at 28°C. Since larval masses enhance their heat output with increasing size, it is suggested that future studies look at what the thermal maxima could be for these larvae as there may be merit in investigating the energy output and ways of harnessing it from containers during the lifecycle.

To limit the environmental effects of feed depth and particle size, it is recommended that *H. illucens* be fed at 5 cm depths with particle sizes less than 5 mm in size so allow for optimal access for consumption and survival.

Chapter 3: investigates how changing larval densities can affect life history and the provisioning rations presented to larvae for their feeding period.

The overall conclusion of this chapter was that feeding efficiencies were optimal at 125 mg/larva/day when the number of individuals are changed to adjust provisioning ration in a set feed medium and space. Not only did survival greatly decrease with very high larval densities (i.e. 50 mg/larva/day) but the estimated provisioning ration doubled in most cases by the end of the experiment. It would be of interest

to see if there are temporal differences in a population and to possibly correlate the change in diet composition with any perceived changes in larval feeding ability or life history and genetics.

Additionally, prepupae appeared in greater proportions when less larvae were present in a population showing that intraspecific competition plays a significant role in feeding ability and life history of *H. illucens* larvae. Larvae showed the highest fat contents at the highest densities, while the inverse was true for protein. This means that it is possible to manipulate the larval nutritional composition simply by adjusting their competition for food. This may also hint at compensatory feeding in the animals and may need further investigation to validate this.

While waste reduction showed similar optimal values above 125 mg/larva/day, but only an optimum at 125 mg/larva/day for bioconversion, it is suggested that there is a limit for bioconversion at 125 mg/larva/day that reverses when larval densities get too low. The same is not true for waste reduction which only reaches a carrying capacity seen up to 200 mg/larva/day. It would be of interest to see if there are negative limits to waste reduction potential in this species above 200 mg/larva/day.

Finally, larval mass appears to have not reached an optimal at 200 mg/larva/day, as this increased from 50-200 mg/larva/day, and it would therefore be of future interest to find out what the maximum, average larval mass would be with change provisioning rate.

Therefore, it is recommended that larvae be kept at densities of 125 mg/larvae/day to produce larvae for harvest where most larvae survive and feed well growing to an economically viable size.

Chapter 4: identified some of the changes that occur in feeding efficiencies and larval size when the size of a population is up-scaled in a single container.

The main conclusions drawn from this chapter is that larvae do not have a carrying capacity for bioconversion when populations are increased in a single container. Therefore, it may be necessary to see if populations can be reared together in hundreds of thousands of individuals per container at a time without negative effects on bioconversion values.

Crawl-off larvae was also seen to be inversely proportional to population size and bioconversion thereby reducing bioconversion when crawl-off was high. This is not ideal as feed does not get processed as it potentially could have been without the larvae crawling off. Therefore, it may be important to identify when and why larvae crawl-off from their feed prior to prepupariation crawl-off.

However, with this increase in population sizes it may lead to impractical and/or expensive harvesting methods, as was seen for the 50 000 larvae treatments which were quite time-consuming, and may need future refining for cleaner harvest. Additionally, waste reduction may reduce dramatically as is seen between 5 000 and 50 000 larvae treatments if taken higher. These are only precautions and it is believed there is merit in looking further at this possible increase in larval population size.

Surface-area-to-volume ratio also increased with increasing population size as expected but density remained similar throughout all containers. What was interesting is the amount of surface area exposed to the atmosphere and its potential link to feeding ability or survival in differently dimensioned feeding setups.

Therefore, it is recommended that larvae be kept either in populations of 5 000-50 000 individuals per container as this constitutes larger populations and less labour and raw material costs with optimal feeding efficiencies.

5.1 Concluding Remarks and Management Recommendations

Larvae should be fed at depths no greater than 10 cm if good growth and feeding ability is required; for faster growth, 5 cm is recommended. Larvae should also always be given homogenised food with a maximum particle size of 5 mm to maximize feeding efficiency and remove the risk of feeding on one nutrient specific food (e.g. the inside an apple they may be able to enter at one opening in the skin).

Larvae should also be fed at densities of 125 mg/larva/day to produce nutritionally well-balanced, good sized larvae with maximum survival.

PRC and OBD are were introduced as new measures of larval feeding efficiencies and life history for *H. illucens*. It is recommended that they be used as primary measures for feeding efficiencies in the future after mathematical justification and refinement.

ADDENDUM

Appendix 1: Glossary of Terms

Ad libitum	To give or do something at the authors' discretion.
Antimicrobial/Antifungal	Reductive or repellent to fungus or bacterial species.
Bioconversion	The conversion of organic matter from one form to another through an organism's consumption in most cases.
Biodiesel	A modified source of energy for machinery derived from biological organisms.
Biomass gain	The net mass gained to an individual, or group of individuals, after consumption of feed.
Coffee pulp waste	Waste from processing of coffee beans.
Control agent	An organism that repels or reduces the occurrence of nuisance or invasive species when present in the same environment.
Crawl-off	The process of larvae (usually of Diptera) which exit a feeding medium to seek a site for prepupariation or pupation. Usually marks the end of feeding for the individual.
Feed accessed	The percentage of feed that was occupied by larvae and thus accessible for consumption.
Feed depth	The overall depth of feed provisioned to larvae measured from the bottom of the container to its' maximum height.
Feed depth lost	The next change in maximum feed height of the diet.
Ecdysis	The moulting of larvae to reach either a new instar or life event and ultimately grow larger.

Efficiency of conversion of digested feed	The amount of feed consumed that is turned into actual body tissue of the organism who consumed it.
Efficiency of conversion of ingested feed	The amount of feed consumed by an organism irrespective of its use thereafter.
En masse	In a group.
Exuviae	The shed 'skin' of an organism following successful ecdysis.
Feeding efficiencies	The ability of an organism to feed, metabolise or bioconvert feed presented to it.
Feeding rate	The average amount of food eaten by an organism over a certain time frame.
Final larval mass	The last instar of larva before prepupariation.
Gram-negative bacteria	A group of bacteria which contain a thin peptidoglycan cell wall and thus don't stain with the addition of crystal violet. These bacteria are regularly anaerobic in nature when rod-shaped.
Harvesting	The collection and processing of organisms at specific time frames for product creation.
Homoscedasticity	When all random variables in a group are within the same finite variance.
Instar	Each stage of larval growth between moults before prepupation.
Intraspecific competition	Competition between individuals of the same species for resources in the same environment.
Larval aggregation heat	The cumulative metabolic heat produced by dipteran larvae when *en masse*.

Larval density	The number of larvae in a given spatial dimension.
Lekking sites	Male aggregation sites for mating with females.
Life history	The changes an organism undergoes during its lifetime.
Lifecycle	The duration of an individual's life from egg to egg.
Mass rearing	The intentional production of organisms in groups for specific goals.
Normality	The fit of a dataset to that of a normally distributed population.
Optimal bioconversion deficit	The percentage realised of a population to reach its optimal ability to bioconvert.
Organic waste	An organic material that is decaying or is no longer edible by human consumption standards.
Oviposition	The deposition of insect eggs by a female's ovipositor.
Pit latrines	A non-water based toilet that collects human excrement. Common in rural areas and informal settlements.
Population size	The number of individuals within a given population.
Power analysis	A statistical method for measuring the degrees of confidence in a dataset and its' given sample size.
Provisioning ration	The amount of food presented to an organism or population but not necessarily ingested or processed.
Provisioning ration change	The difference in the calculated provisioning ration at two separate points of an experiment.

Proximate Analysis / Composition	Identification of macronutrients and other variables of feeds important to feeding efficiencies of an organism. These are commonly crude protein, lipids, moisture content, crude fibre and ash content.
Survival	The number of individuals that were found alive after a certain period of time.
Treatment	The independent variable investigated/manipulated during an experiment.
Trial	A period of time under which an experiment takes place within.
Waste reduction	The amount of organic material that is removed from the feed after a set period of feeding by an organism.
Wet Separation	A method of harvesting using water and sieves to separate organisms from left over residue.

Appendix 2: Proximate Analyses Methods & formulae used in this thesis

All proximate analyses in this thesis were performed through Quantum Analytical Services (Malmesbury, South Africa) and each component of it was done according to the following methods:

Crude Fibre

Protein, fat, starch and other digestible carbohydrates are removed from the sample by hydrolysis with hot acid and alkali. The residue is dried and ashed to determine the loss on ignition of the crude fibre. Reference: ANKOM, AOCS Ba 6a-05

Ash Content

The sample organic matter is heated in a muffle furnace to remove organic material. The remaining residue of mineral ash is determined by differential weighing before and after heating. Reference: AOAC 942.05, ALASA 2.5.1

Moisture Content

Loss of mass of a test portion of the sample on drying is determined under specified conditions. ISO6496:1999(E) filter bag. Reference: ANKOM, AOCS Am 5-04

Crude Fat

Crude fat is determined using the ANKOM XT15 extraction system and measuring the loss in mass after extraction with petroleum ether of fat or oil from the sample encapsulated in a filter bag. Reference: ANKOM, AOCS Am 5-04

Crude Protein

The sample is combusted at 900°C in the presence of pure oxygen to nitrogen, carbon dioxide and water. The nitrogen is measured by a thermal conductivity detector after selective removal of the carbon dioxide

and water. A factor based on sample type is used to convert the nitrogen to crude protein. Reference: AOAC 992.23/990.03

CHOs (Sugars and Starches – i.e. Carbohydrates)

The sum total of Ash, crude fibre, moisture, crude protein and crude fat were calculated and the difference from 100 was determined to be sugars and starches. Reference: Unknown???

For all studies in this thesis standard calculations and methods were used as follows for the respective results of each:

Moisture content was determined as follows: 1) 5g samples were weighed out and dried at 80°C for 24 h and then weighed again and replaced to dry again for a further hour, if the difference between the two final weights was < 0.5% (if not, this process was repeated until the aforementioned was achieved), then the moisture content was calculated as follows:

$$Moisture\ content\ (\%) = \frac{(wet\ weight\ (i) - dry\ weight\ (f))}{wet\ weight\ (i)} \times 100$$

Waste reduction bioconversion and efficiency of conversion of digested-feed (ECD) were determined from dry matter (DM) using the following formulae adapted from Scriber & Slansky (1981):

$$Waste\ reduction = \frac{(wt\ (i) - wt\ (f))}{wt\ (i)} \times 100\%$$

$$Bioconversion\ = \frac{(larval\ biomass\ (f) - larval\ biomass(f))}{food\ mass\ (i)} \times 100$$

$$Efficiency\ of\ conversion\ of\ digested\ feed = \frac{larval\ biomass\ gained}{food\ (i) - food\ (f)}$$

A change in provisioning ration was calculated by calculating provisioning ration at the beginning and end of the experiments for each replicate (see *Provisioning ration* equation). The difference of these two was compared to the initial rate to understand the dynamics of how Provisioning ration changed and is affected by population density. The formula is:

$$Provisioning\ ration = \left(\frac{diet\ mass}{no.\ larvae}\right) \div trial\ duration$$

$$Provisioning\ ration\ change = \frac{(provisioning\ ration\ (f)\ - provisioning\ ration(i))}{provisioning\ ration(i)}$$

A theoretical bioconversion was also calculated using the average individual weights of larvae (estimated from total biomass per replicate and the initial/final number of larvae) to estimate a theoretical initial and final biomass and the difference between theoretical and realised bioconversion was calculated as the optimal bioconversion deficit (OBD). The formula is as follows:

$$Average\ Larval\ Weight\ = \frac{total\ biomass}{no.of\ individuals}$$

$$Optimal\ Bioconversion\ = \frac{(avg\ larval\ wt \times larvae\ (f)) - (avg\ larval\ wt \times larvae\ (f))}{feed\ (i)}$$

$$Optimal\ Bioconversion\ Deficit\ =\ Optimal\ bioconversion - bioconversion$$

The differences between the temperatures of the aggregations and ambient air were also calculated daily over the experimental phase as well as the percentage of total feed depth lost and the percentage of feed accessed by larvae, as follows:

$$Aggregation\ temp\ difference = Aggregation\ temperature - Ambient\ temperature$$

$$Total\ depth\ lost = final\ diet\ depth\ /\ initial\ diet\ depth$$

$$Volume\ of\ diet\ accessible = \frac{\pi \times radius^2 \times larval\ depth}{max\ diet\ depth}$$