Stocking rate and organic waste type affect growth and body composition of three *Chrysomya* species and *Lucilia sericata* (Diptera: Calliphoridae): implications for bioconversion

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Abstract

Fly larvae can be used effectively to reduce various organic waste types and produce value-added products, including protein as an ingredient in livestock feeds and oil for biodiesel production. However, fly development on different waste types may cause differences in growth rate and the body composition, which can further be influenced by fly species and their stocking rate. This study explored the impact of different waste types (kitchen waste, abattoir waste and swine manure) and larval stocking rate on growth and body composition of four blowfly species, *Chrysomya chloropyga* (Wiedemann), *Chrysomya megacephala* (Fabricius), *Chrysomya putoria* (Wiedemann) and *Lucilia sericata* (Meigen). First instar larvae (20, 50 or 100), less than 3 hours old, were placed on 100 g of each waste type. Pre-pupal mass at commencement of post-feeding larval dispersal, time to onset of dispersal, survival and nutrient reserves were determined for each species, stocking rate and waste type. Our results revealed that larvae fed kitchen and abattoir waste had significantly higher dry mass, crude protein and lipid content compared to those fed swine manure. Higher survival rate was observed with increasing larval stocking rate. We provide important information to guide the mass production of high-quality nutrient-rich larvae and recommend *C. putoria*, which is versatile and effective on a range of waste products, as well as high in protein and lipids. The implications for waste management are discussed.

Keywords:

Bioconversion; Stocking density; Larval development; Survival; Nutrient reserves; Waste valorisation

1. Introduction

There are substantial benefits and commercial potential for using flies for bioconversion (Sánchez-Muros et al., 2014). Fly larvae can reduce and recycle plant waste (Tschirner & Simon, 2015), poultry, dairy, beef and swine manure (Larraín & Salas, 2008; Wang et al., 2013), fecal sludge (Diener et al., 2011; Banks et al., 2014), fish renderings (Nguyen et al., 2013), meat processing waste (Yehuda et al., 2011), brewery waste (Tschirner & Simon, 2015) and leftover coffee grounds (Lardé, 1990). The common house fly, *Musca domestica* (L.) (Muscidae) and the black soldier fly, *Hermetia illucens* (L.) (Stratiomyidae), are the two main species that have been extensively studied for use in bioconversion (Makkar et al., 2014; Čičková et al., 2015). *Musca domestica* has been reared successfully on manure and meat waste. However, this species is a well-known pest across the world and experiences high mortality on manure (Larraín & Salas, 2008). Black soldier flies have atrophied mouth parts and are not attracted to people, food or buildings and consequently are not mechanical vectors of disease and are not considered a pest species (Čičková et al., 2015). The larvae of *H. illucens* have been reared successfully on a wide range of organic waste, with particular success on plant waste and manure (Wang et al., 2013; Tschirner & Simon, 2015). Despite the number of studies that have looked into these two fly species, they are not necessarily the best suited flies for bioconversion of all waste products. *Hermetia illucens* does not grow well on waste containing high levels of meat or animal fat (Nguyen et al., 2013). Additionally, it can be challenging to successfully breed *H. illucens* under artificial lights in laboratory conditions (Zhang et al., 2010) and the development time from egg to adult takes more than a month, making this species an economically difficult species to breed (Tomberlin et al., 2009).

The different types of organic waste that can be broken down by flies are highly variable in nutrient content. These differences lead to large variation in larval body composition within the same species. The protein content of *H. illucens* can range from less than 20% to slightly higher than 50% when fed on different organic waste types, with an average of 40 – 45% (Table 1). The lipid content of *H. illucens* is even more variable, ranging from 3.4% to 40%. The protein content that has been recorded for *M. domestica* larvae and pupae reared on different waste types varies between 29% and 77% (Table 1). Larval body composition can also vary according to differences in waste across regions (Zhou et al., 2013). However, caution is required when comparing larval body composition across different studies due to differences in analytical methods (Tschirner & Simon, 2015; Pieterse & Pretorius, 2013), the

Table 1. Nutritional composition on a dry matter basis of different species of fly larvae (L) or pupae (P) reared on different organic waste types

Species	Substrate	Larvae/Pupae	CP (%)*	Lipids (%)	Ash (%)	Study
H. illucens	Sugar beet pulp	L	52.3	3.4	22.9	Tschirner & Simon 2017
	Meat and bone meal	L	45.2	21.1	11	Pastor *et al.*, 2015
	Meat and bone meal	L	44.6	34.2	12.4	Kyntäjä *et al.*, 2014
	Distillers grains	L	44.6	38.6	4.8	Tschirner & Simon 2015
	Swine manure	L	43.6	33.1	15.5	St-Hilaire *et al.*, 2007b
	Swine manure	L	43.2	28.0	-	Newton *et al.*, 2005
	Cattle manure	L	42.1	34.8	7.0	Newton *et al.*, 1977
	Poultry manure	L	40	18-28	-	Bondari and Sheppard 1981
	Poultry manure	L	36.9	18.8	17.4	Arango *et al.*, 2004
	Cereal processing waste	L	30.2	37.2	13.5	Tschirner & Simon 2016
	Citric waste	L	25.4	1.5	19.5	Pastor *et al.*, 2015
	Brewery waste	L	22.7	29.2	6.6	Pastor *et al.*, 2015
	Olive waste	L	17.7	40.8	10.9	Pastor *et al.*, 2015
M. domestica	Bran and blood	P	76.23	14.39	15.7	Pieterse & Pretorius 2014
	Cow manure	P	70.4	16.1	9.8	St-Hilaire *et al.*, 2007a
	Bran and blood	L	60.38	14.08	8.59	Pieterse & Pretorius 2013
	Poultry manure	L	59.5	6.7	11.53	Dordevic *et al.*, 2008
	Swine manure	L	56.9	23.8	8.6	Wang *et al.*, 2013
	Poultry manure	L	43.4	14.3	14.3	Fasakin *et al.*, 2003
	Poultry manure	L	28.6	23.3	29.6	Ogunji *et al.*, 2008
C. megacephala	Meat processing waste	L	61.8	27	7.2	Barroso *et al.*, 2015
	Meat processing waste	P	46.8	16.5	6.1	Barroso *et al.*, 2015
L. sericata	Meat processing waste	P	59	26.6	4.9	Barroso *et al.*, 2015
	Fish and poultry	P	57.5	23.6	11.2	Yehuda *et al.*, 2012
	Meat processing waste	L	53.5	28.4	4.9	Barroso *et al.*, 2013
	Fish and poultry	L	51.8	32.5	10.7	Yehuda *et al.*, 2011

*CP: Crude Protein

age of the larvae (Aniebo & Owen, 2010; Barroso et al., 2014; Pieterse & Pretorius, 2013), and the manner in which they were processed (Aniebo & Owen, 2010; Pieterse & Pretorius, 2013).

The stocking rate of fly larvae (i.e., the number of individuals introduced) on a substrate is important for larval survival and the effective reduction of the substrate. At lower stocking rates there is reduced competition between larvae, leading to greater larval mass and survival and greater nutrient assimilation per larva (Diener et al., 2009), which in turn influences eclosion rate, adult survival and reproductive success (Tomberlin et al., 2002; Nestel & Nemny-Lavy, 2008). However, when larval stocking rate is too low there is little reduction of the substrate, which is inefficient in a facility attempting to reduce waste and produce protein (Barnard et al., 1998; Diener et al., 2009). Increasing larval stocking rate can introduce strong competition for nutrients between larvae, but it can also promote larval survival. Increased stocking rate can elevate heat accumulation, leading to more rapid larval development, more efficient assimilation of the substrate through mass release of digestive enzymes, and reduced chance of desiccation (Anderson, 2010; Rivers et al., 2011; Kotzé et al., 2015). Extensive study is required to determine the effects of high larval stocking rate on larval survival and body composition, and the effective reduction of the substrate or waste product for a bioconversion facility to be efficient.

The aim of this study was to determine the effects of species, larval stocking rate and organic waste type on the body mass, survival, duration of larval development and body composition (dry mass, protein, carbohydrate, lipid, and water content) of four blow fly species (Diptera: Calliphoridae). The species that were chosen for this study were *Chrysomya chloropyga* (Wiedemann), *Chrysomya megacephala* (Fabricius), *Chrysomya putoria* (Wiedemann) and *Lucilia sericata* (Meigen) (Calliphoridae). All four species are associated with carrion and are used by forensic entomologists to estimate time of death (Williams & Villet 2006a, Richards et al. 2009a). Two of these species, *C. megacephala* and *C. putoria*, are also associated with manure (Laurence 1988; Lindsay et al. 2013). Consequently, these fly species have the potential to be used for bioconversion of a number of different wastes, especially manure and meat-processing waste. All are present in Gauteng province, South Africa (Parry et al., 2016) where this study was conducted. *Chrysomya chloropyga* and *C. putoria* have a wide distribution across Africa (Richards et al., 2009b), while *C. megacephala* was introduced into South Africa from south-east Asia and east Africa (Williams & Villet, 2006b) and *L. sericata* is present in northern Europe, Australia and South Africa (Zumpt, 1965; Smith & Wall, 1997;

Richards et al., 2009a). They are known to be highly prolific, have a short larval development time and are easy to rear under laboratory conditions. The three waste types that were tested in this study were kitchen waste, swine manure and meat-processing waste (referred to as abattoir waste). These waste types are readily available and often problematic or expensive to remove and process, but are high in nutrients that can be recovered by fly larvae.

2. Materials and methods

2.1. Colony Establishment

Adult blowflies were collected from various areas around Gauteng, South Africa, using modified Red-Top® hanging traps (Miller Methods, Ltd., Pretoria) as described in Parry et al. (2016). Traps were hung from trees and were checked daily until the appropriate fly species and numbers (between 20 and 100) had been collected. Additional flies were caught throughout the duration of the study to maintain colony fitness and genetic variability. Flies were identified using available taxonomic keys (Zumpt, 1965; Rognes & Paterson, 2005; Williams & Villet, 2014).

Live, identified flies were kept in single species rearing cages. The cages were constructed from 13-litres transparent plastic containers (MaxiMultiBox, Hobbylife, Istanbul, Turkey) with dimensions of 0.36 × 0.25 × 0.23 m (0.0207 m^3 /20 L). A 0.2 × 0.1 m^2 section was cut from the lid of the plastic container and covered with white voile fabric for ventilation. One side of the cage had a circular hole (diameter: 15 cm), fitted with a 45 cm sleeve of white voile fabric to allow daily feeding, removal of eggs and pupae, and cleaning of the cage. Flies were provided with unrestricted access to sugar, milk powder as a protein source, and water from soaked cotton wool. The rearing cages were maintained at 25 ± 2 ˚C in a room with a window to provide a natural day:night light cycle. Food-grade chicken liver was provided in a 150 mL plastic container (WestPack Lifestyle) as an egg-laying substrate to maintain the cultures. A larger 500 mL plastic container (WestPack Lifestyle) with sterile river sand was placed underneath the breeding medium to be used by the flies for pupation.

2.2. Larval feeding and nutrient assimilation

2.2.1. Assessment of bioconversion

Each species was trialled on three different waste products: pre-consumer food wastage collected from a green-grocer (Food Lover's Market, Johannesburg, South Africa), abattoir waste (Chamdor Meat Packers, Krugersdorp, South Africa), and fresh, unprocessed swine manure collected from pig farms present on the University of Pretoria Onderstepoort Campus. The manure was collected from non-lactating swine that had not received antibiotics or anti-parasitic treatments prior to manure collection.

Egg laying was carefully monitored and freshly hatched first-instar larvae (less than three hours old) were collected from rearing cages using a fine camel-hair paint brush dipped in distilled water to easily pick up the larvae, a total of 20, 50 or 100 larvae were placed on 100 g of each waste product. Freshly hatched larvae were used instead of eggs due to the possibility of eggs not hatching and the difficulty of counting eggs accurately from a clutch without damaging them. Larvae were provided with 5g/larva, 2g/larva or 1g/larva of waste product to manipulate larval stocking density. Waste products were weighed using an analytical balance (to 0.0001 g; AS220/C/2, Radwag, Poland). Three batches of each waste product collected at various times were used, with five replicates from each batch that were run simultaneously for each species. This level of replication encompassed some of the variability resulting from the quality of different batches of the selected waste products. The cups containing each replicate of the waste product were maintained at 28˚C in an incubator (LTIE, Labcon, Gauteng, South Africa) with a 12 hour L:D cycle. Five larvae were randomly selected, weighed and returned to the waste product every 12 hours until they began to wander. This dispersal behaviour is exhibited by Calliphoridae fly larvae when they enter a pre-pupal phase, which is characterised by a cessation of feeding and movement away from the feeding substrate in search of a suitable place to pupate (Richards et al., 2009a). Following this, the pre-pupal larvae were removed from the medium and were frozen and stored at -70˚C for later freeze-drying and biochemical analysis.

2.2.2. Body composition of pre-pupal larvae

Body composition of individual pre-pupal larvae fed on the different waste products were determined. The methods described by Foray et al. (2012), which are based on van Handel's method (van Handel, 1985a,b) estimate total dissolvable protein, lipid and carbohydrate

content in the same individual insect. Water content of pre-pupal larvae was determined using gravimetric methods (e.g., Weldon et al., 2016). A detailed description of these methods, as well as their strengths and weaknesses, are presented in Appendix A.

2.3. Data analysis

Our general statistical approach was to run separate models for each waste type to determine the effects of species and initial larval stocking rate. Batch was included in these models as a random effect. This was the most appropriate means of analysis because waste types were tested at different times. After these analyses, waste types were analysed in a single model to determine potential differences between waste types on dependent variables. In these models, batch as a random effect was not included. The results of these models need to be treated cautiously, but in cases where waste type was significant, the effect sizes (based on mean square) were large.

General linear models with predictors as described above were run for mass of pre-pupal larvae and time to dispersal. General linear models were also used to assess the effects of species, initial stocking rate and waste type on estimated total protein, carbohydrate and lipid content of individual larvae. Dry mass was included as a covariate in these models. The water content of pre-pupal larvae was also assessed and compared across species, stocking rate and waste type using a general linear model, with wet mass as a covariate. Tukey's honestly significant difference tests were used for post-hoc multiple comparisons. All general linear models were run in SPSS Version 23 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0).

A logistic regression was run in RStudio version 0.99.903 running R version 3.3.1 (R Development Core Team, 2016) using the command "cbind" to combine the number of alive and dead larvae to assess the effects of species and initial larval stocking rate on the proportion of larvae that survived to reach the post feeding dispersal phase. As with the linear models described above, logistic regression analyses were run using the data from each waste type separately; and then across all three waste types at once to determine the effect of waste type on survival of larvae.

3. Results

3.1 Mass at onset of dispersal phase

For kitchen waste, there was no significant effect of stocking rate, species, their interaction, or batch on pre-pupal mass (Figure 1a; Table 2). For larvae fed on abattoir waste, there was a significant effect of species, where *C. chloropyga* were heaviest and *L. sericata* had the lowest mass (Figure 1b; Table 2). Batch also explained significant variation in mass on this waste type. The effect of stocking rate and the interaction of stocking rate and species on pre-pupal mass were not significant for larvae fed on abattoir waste. Pre-pupal mass of larvae fed on swine manure was significantly affected by species and stocking rate (Figure 1c; Table 2), but there was no significant effect of the interaction of stocking rate and species. *Chrysomya megacephala* and *C. putoria* were heavier in comparison with *C. chloropyga* and *L. sericata*. When fed swine manure, larvae at an initial stocking rate of 20 were heavier than larvae at an initial stocking rate of 100 per 100 g of waste. Mass was lowest at a stocking rate of 100, except for *C. chloropyga*, where mass was lowest among larvae at a stocking rate of 50 per 100 g of waste.

Table 2. Summary results from a general linear model showing the effects of species, initial larval stocking rate and waste type on mass of pre-pupal larvae

Waste	Effect	Mean Square	*F*	d.f	*p*
Kitchen	Species	14811.992	1.456	3	0.317
	Stocking rate	2835.288	4.458	2	0.096
	Batch	14546.768	1.502	2	0.303
	Species*Stocking rate	1122.923	0.997	6	0.470
	Error				
Abattoir	**Species**	**68823.701**	**60.364**	**3**	**0.0001**
	Stocking rate	396.150	0.976	2	0.452
	Batch	**9232.722**	**6.981**	**2**	**0.023**
	Species*Stocking rate	222.427	0.995	6	0.471
	Error				
Swine Manure	**Species**	**21672.845**	**27.788**	**3**	**0.001**
	Stocking rate	**3005.796**	**8.577**	**2**	**0.036**
	Batch	**8694.779**	**13.024**	**2**	**0.034**
	Species*Stocking rate	347.168	0.750	6	0.621
	Error				
All Waste	**Species**	**31181.002**	**8.312**	**3**	**0.009**
	Stocking rate	274.096	0.782	2	0.510
	Waste	**11509.070**	**44.694**	**2**	**0.006**
	Species*Stocking rate	268.864	0.327	6	0.913
	Species*Waste	**32438.319**	**7.592**	**6**	**0.004**
	Stocking rate*Waste	**3101.607**	**5.513**	**4**	**0.033**
	Species*Stocking rate*Waste	732.165	1.757	12	0.136
	Error				

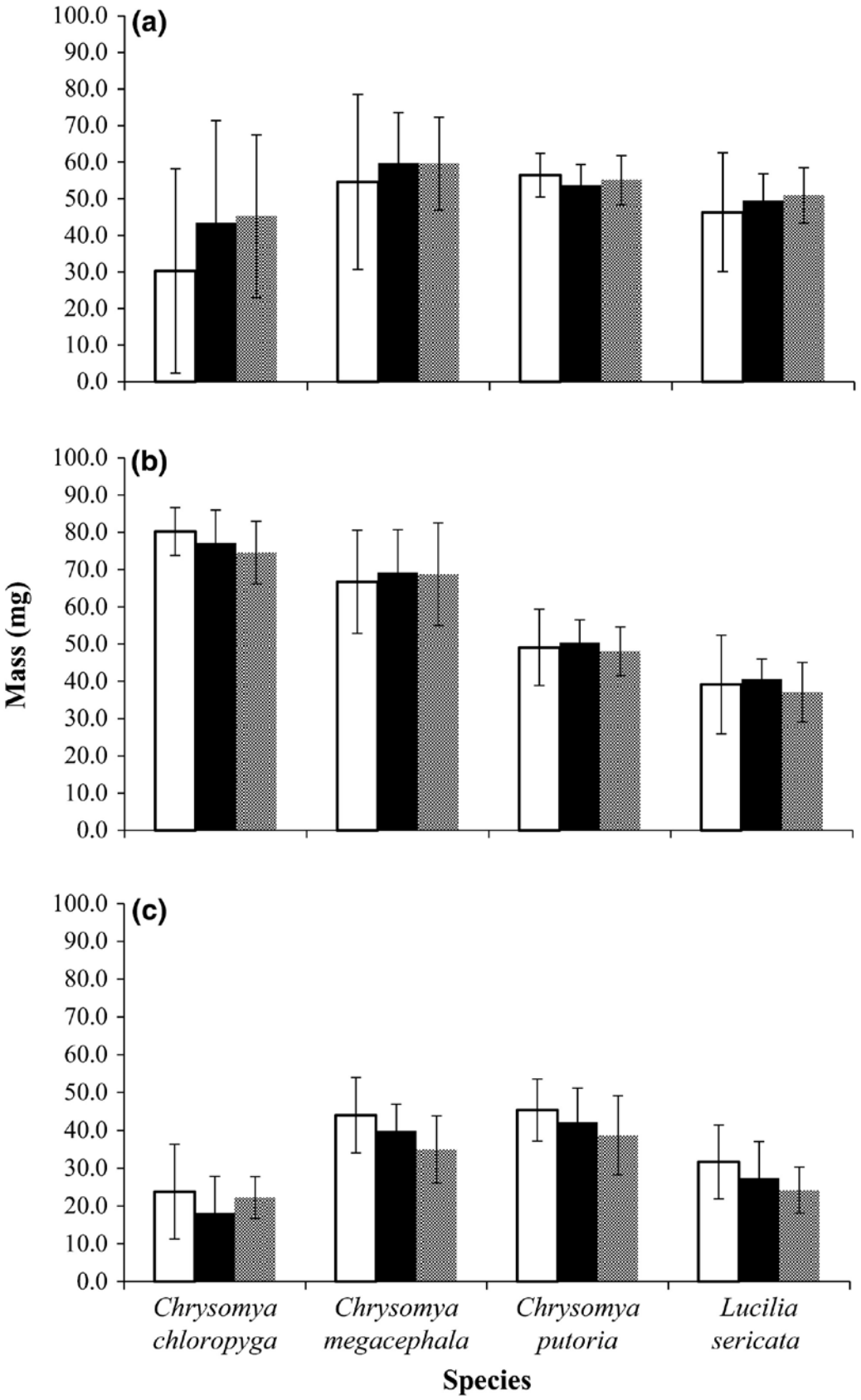

Figure 1. Mass of larvae at onset of dispersal for four calliphorid species held at different stocking rates. Error bars indicate ±1 *SE*. (a) Kitchen waste, (b) abattoir waste (c) and swine manure. Different initial larval stocking rates are represented by different bars. White bar: 20, black bar: 50, dotted bar: 100

When comparing across waste types, there was a strong effect on pre-pupal mass, and an interaction between species and waste type (Table 2). When fed on abattoir waste, pre-pupal larvae of *C. chloropyga* were heaviest, while pre-pupal larvae of the same species had the lowest mass when fed on swine manure. There were also interactions of waste type with stocking rate, and a three-way interaction of waste type with species and stocking rate (Table 2). There was a trend for pre-pupal mass to decrease with an increase in stocking rate, with the exception of species fed on kitchen waste, where there were no differences across stocking rates. There was also a difference in this trend in *C. megacephala* fed on abattoir waste where there was no difference across the different stocking rates, and these did not differ from *C. chloropyga* fed on swine manure.

3.2 Mean time to onset of dispersal phase

There was no significant effect of species or stocking rate on the mean time to onset of dispersal when larvae were fed on kitchen waste (Figure 2a; Table 3), abattoir waste (Figure 2b; Table 3) or swine manure (Figure 2c; Table 3). There was significant variation present across different batches for abattoir waste but not for kitchen waste or swine manure. Based on visual observations of the waste that was received in this study, the abattoir waste was fattier in the second and third batch, and the third batch appeared to have more intestine than in the first batch, while swine manure was more homogenous over all three batches. There was a significant species by stocking rate interaction on mean time to onset of dispersal among larvae fed swine manure. *Chrysomya chloropyga* began dispersal sooner at an initial stocking rate of 20 and 50 than at a stocking rate of 100 larvae per 100 g of waste. The other three species did not differ in time to dispersal across the different stocking rates.

Overall, no differences were found across all three waste types, but there were species by stocking rate interactions and species by stocking rate by waste interactions. *Chrysomya chloropyga*, with an initial stocking rate of 20 larvae per 100 g of waste reached the dispersal phase sooner than any other species when fed on kitchen waste, followed by swine manure. This species also exhibited large variation in the time it took to reach dispersal when fed on kitchen waste, especially for pre-pupal larvae held at a low initial stocking rate of 20 larvae per 100 g of waste. This large variation was also present in *C. megacephala* and *L. sericata* at a stocking rate of 20 larvae per 100 g of waste. *Chrysomya chloropyga* held at initial stocking rates of 20 and 50 larvae per 100 g of waste had a significantly lower time to onset of dispersal than all other species when fed on swine manure.

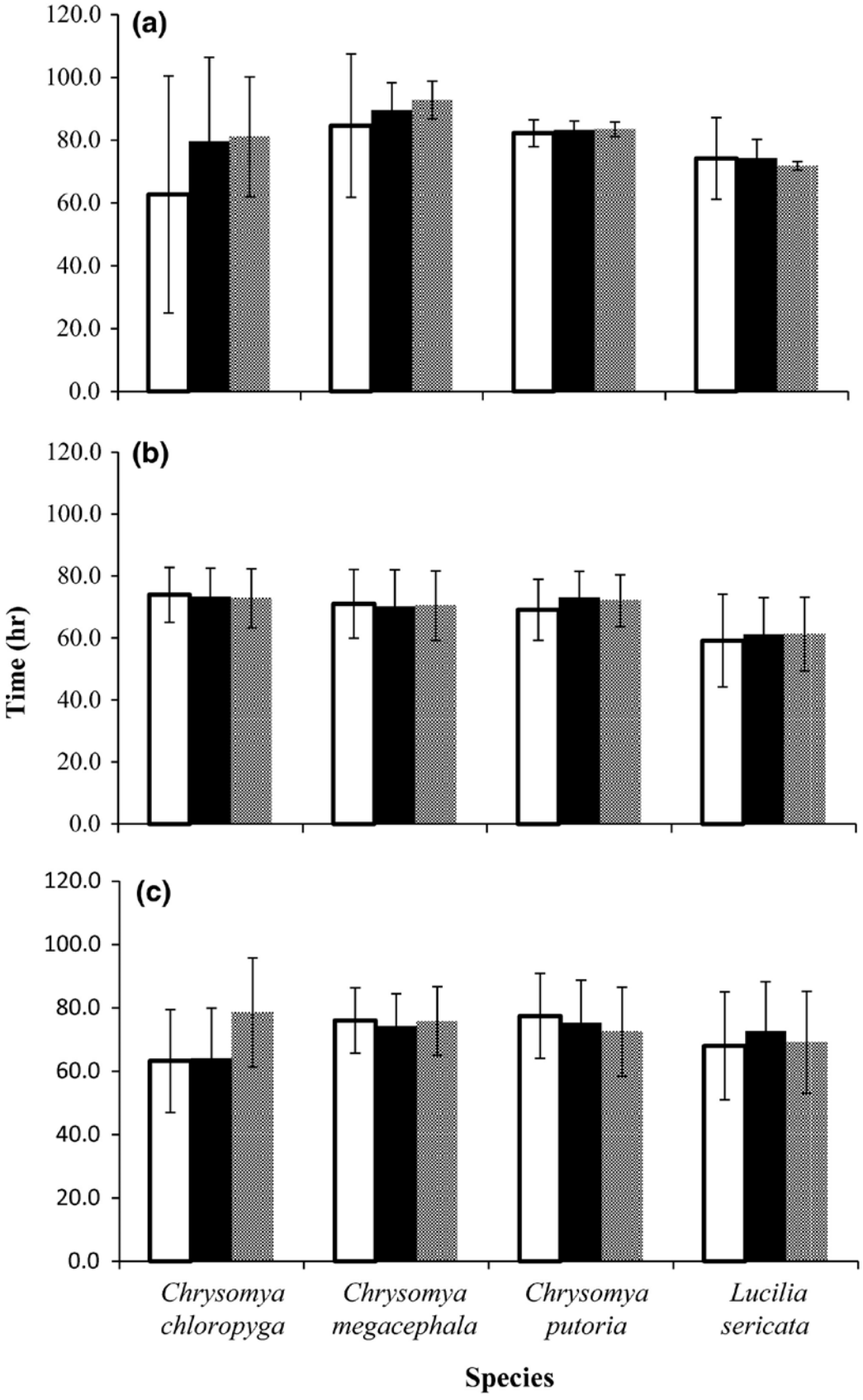

Figure 2. Mean time to the onset of dispersal of larvae of four calliphorid species held at different stocking rates. Error bars indicate ±*SE*. (a) Kitchen waste, (b) abattoir waste and (c) swine manure. Different initial larval densities in 100 g of waste are represented by different bars. White bar: 20, black bar: 50, dotted bar: 100

Table 3. Summary results from a general linear model showing the effects of species, initial larval stocking rate and waste type on time to dispersal of pre-pupal larvae

Waste	Effect	Mean Square	*F*	d.f	*p*
Kitchen	Species	12224.587	1.951	3	0.223
	Stocking rate	3720.160	5.967	2	0.063
	Batch	3547.360	0.576	2	0.592
	Species*Stocking rate	1863.947	2.546	6	0.079
	Error				
Abattoir	Species	7371.307	3.021	**3**	0.116
	Stocking rate	112.000	0.852	2	0.492
	Batch	**17810.080**	**7.215**	**2**	**0.025**
	Species*Stocking rate	117.547	1.144	6	0.395
	Error				
Swine Manure	Species	2708.525	0.326	3	0.807
	Stocking rate	699.178	0.793	2	0.513
	Batch	14625.628	1.673	2	0.259
	Species*Stocking rate	**1940.464**	**4.252**	**6**	**0.016**
	Error				
All Waste	Species	9670.422	1.579	3	0.270
	Stocking rate	2021.915	3.452	2	0.111
	Waste	27434.919	4.457	2	0.126
	Species*Stocking rate	**1286.902**	**3.088**	**6**	**0.035**
	Species*Waste	3314.659	0.627	6	0.707
	Stocking rate*Waste	1051.713	2.057	4	0.205
	Species*Stocking rate*Waste	**1442.052**	**3.249**	**12**	**0.012**
	Error				

3.3 Survival

There was a significant effect of species (Logistic Regression: $\chi2 = 914.23$, df = 3, $p < 0.001$), stocking rate (Logistic Regression: $\chi2 = 113.58$, df = 2, $p < 0.001$), and their interaction (Logistic Regression: $\chi2 = 96.47$, df = 6, $p < 0.001$) on survival of larvae fed kitchen waste (Figure 3a). *Chrysomya chloropyga* had the lowest survival, especially at a stocking rate of 20 larvae per 100 g of waste. Larvae of *C. megacephala* and *C. putoria* had high survival, with their highest survival at a stocking rate of 100 on 100 g of kitchen waste.

On abattoir waste, species had a strong effect on survival to the pre-pupal stage (Figure 3b; Logistic Regression: $\chi2 = 162.89$, df = 3, $p < 0.001$) where *C. chloropyga* had the highest survival and *L. sericata* had the lowest survival. There were also significant differences across the different stocking rates (Logistic Regression: $\chi2 = 31.74$, df = 2, $p < 0.001$), and a significant species by stocking rate interaction (Logistic Regression: $\chi2 = 109.703$, df = 6, $p < 0.001$). Stocking rate did not affect the survival of *C. megacephala* and *C. putoria*. Larvae of *L. sericata* had low survival at a stocking rate of 20 per 100 g of waste, with an increase in

survival when initial stocking rate increased. Among *C. chloropyga*, lowest survival was observed at the highest stocking rate.

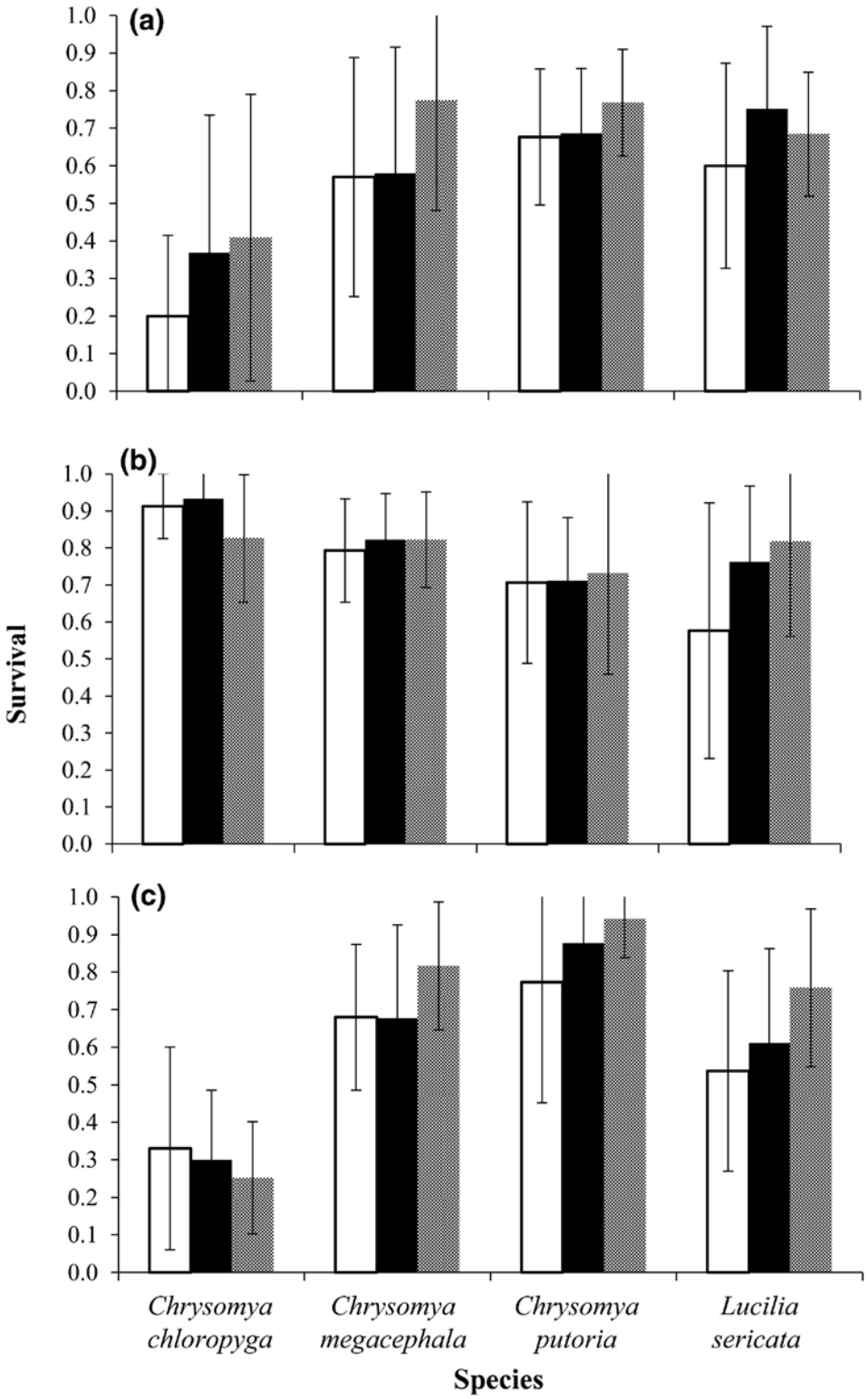

Figure 3. Proportion of larvae that survived until onset of dispersal of four calliphorid species held at different stocking rates. Error bars indicate ±*SE*. (a) Kitchen waste, (b) abattoir waste and (c) swine manure. Different initial larval densities in 100 g of waste are represented by different bars. White bar: 20, black bar: 50, dotted bar: 100

The proportion of larvae that survived on swine manure was significantly different between species (Figure 3c; Logistic Regression: $\chi2 = 2546.84$, df = 3, $p < 0.001$), with *C. putoria* followed by *C. megacephala* having the highest survival. Stocking rate (Logistic Regression: $\chi2 = 112.49$, df = 2, $p < 0.001$) and the interaction of species by stocking rate (Logistic Regression: $\chi2 = 125.32$, df = 6, $p < 0.001$) also affected survival on swine manure. Survival increased with an increase in the initial stocking rate of larvae, except for *C. chloropyga* where survival decreased with an increase in initial stocking rate.

When all three waste types were analysed together, waste type had a significant effect on survival (Logistic Regression: $\chi2 = 943.52$, df = 2, $p < 0.001$), with larval survival highest when they were reared on abattoir waste but lowest on swine manure. There were also species by stocking rate interactions (Logistic Regression: $\chi2 = 133.92$, df = 6, $p < 0.001$), species by waste interactions (Logistic Regression: $\chi2 = 1863.52$, df = 6, $p < 0.001$), stocking rate by waste interactions (Logistic Regression: $\chi2 = 51.40$, df = 4, $p < 0.001$) and species by stocking rate by waste interactions (Logistic Regression: $\chi2 = 197.57$, df = 12, $p < 0.001$). These interaction effects indicate that each species at each stocking rate responded differently to the different waste types. Survival was highest in pre-pupal larvae that were fed on abattoir and lowest in those fed on kitchen waste. Survival tended to be higher for larvae kept at a stocking rate of 100 per 100 g of waste, except where there were no differences in survival across different stocking rates, such as for *C. megacephala* and *C. putoria* feeding on abattoir waste, or where survival was higher at lower stocking rates, such as in *C. chloropyga* fed on swine manure and abattoir waste and *L. sericata* fed on kitchen waste.

3.4 Larval body composition

Protein content of pre-pupal larvae fed on kitchen waste and swine manure was affected by the effect of dry mass (Table 4). In both cases, protein content significantly increased with dry mass (kitchen waste: coefficient estimate = 0.020, S.E. = 0.007; swine manure: coefficient estimate = 0.029, S.E. = 0.009). Regardless of species, protein content was high but similar when pre-pupal larvae developed on kitchen waste. This contrasted with the protein content of pre-pupal larvae reared on swine manure, where there was a species difference. On swine manure protein content of all four species was low, but especially so in *C. chloropyga* and *L. sericata* (Figure 4a). When developing on abattoir waste, the effect of species also had a significant effect on protein content of pre-pupal larvae (Table 4), with protein content of *C. chloropyga* being significantly higher than *C. megacephala*, which in

turn had higher protein content than *C. putoria* and *L. sericata*. These patterns were also evident when all three waste types were included in the same analysis, where there was a significant effect of species and waste type and an interaction between species and waste type (Table 4). Across all species, protein content of pre-pupal larvae fed on kitchen waste was higher than those fed on swine manure. In the analysis including all waste types, there was also an effect of dry mass on protein content, reflecting an increase in protein content as dry mass increased (coefficient estimate = 0.017, S.E. = 0.05).

Table 4. Summary results from a general linear model showing the effects of species, initial larval density and waste type on dissolvable protein content

Waste	Effect	Mean Square	*F*	df	*p*
Kitchen	**Intercept**	**18.094**	**75.640**	**1**	**<0.001**
	Dry Mass	**5.454**	**25.420**	**1**	**<0.001**
	Species	0.148	0.692	3	0.558
	Stocking rate	0.441	2.057	2	0.130
	Batch	0.653	27.627	2	0.903
	Species*Stocking rate	0.177	0.824	6	0.552
Abattoir	**Intercept**	**21.758**	**43.153**	**1**	**<0.001**
	Dry Mass	**1.216**	**4.139**	**1**	**0.043**
	Species	**3.944**	**13.423**	**3**	**<0.001**
	Stocking rate	0.069	0.234	2	0.791
	Batch	**2.922**	**9.946**	**2**	**<0.001**
	Species*Stocking rate	0.304	1.035	6	0.403
Swine Manure	**Intercept**	**10.485**	**31.478**	**1**	**<0.001**
	Dry Mass	**2.204**	**13.400**	**1**	**<0.001**
	Species	**0.550**	**3.345**	**3**	**0.020**
	Stocking rate	0.071	0.432	2	0.650
	Batch	**1.238**	**7.527**	**2**	**0.001**
	Species*Stocking rate	0.151	0.918	6	0.483
All Waste	**Intercept**	**51.576**	**215.778**	**1**	**<0.001**
	Dry Mass	**2.726**	**12.562**	**1**	**<0.001**
	Species	**1.235**	**5.166**	**3**	**0.002**
	Stocking rate	0.110	0.459	2	0.632
	Waste	**5.841**	24.436	**2**	**<0.001**
	Species*Stocking rate	0.425	**1.777**	6	0.101
	Species*Waste	**1.505**	**6.295**	**6**	**<0.001**
	Stocking rate*Waste	0.283	1.184	4	0.316
	Species*Stocking rate*Waste	0.116	0.487	12	0.923

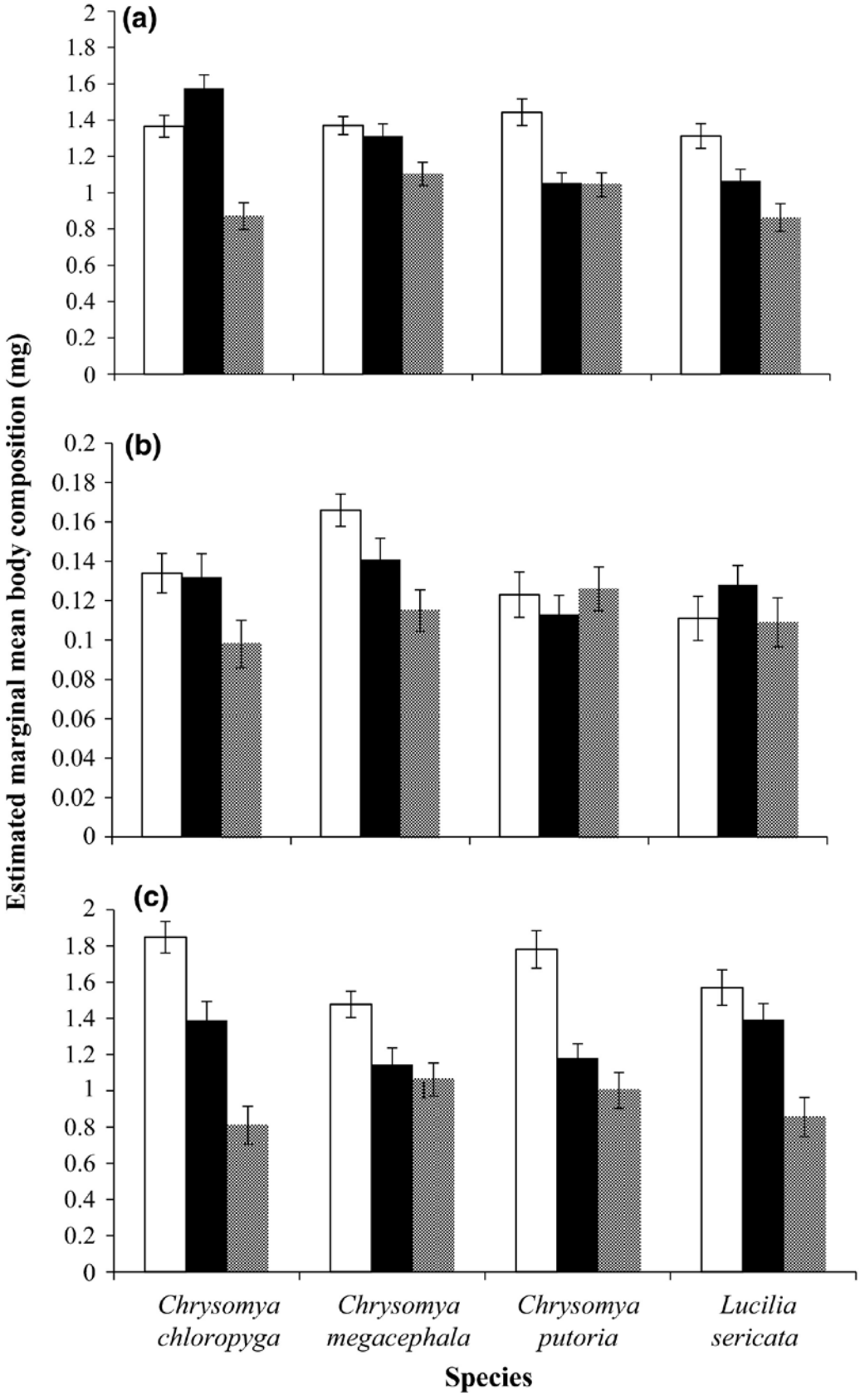

Figure 4. Estimated marginal mean body composition of pre-pupal larvae of four calliphorid species fed on different waste types. Error bars indicate ±1 *SE*. (a) Protein, (b) carbohydrates and (c) lipids. Different waste types are represented by different bars. White bar: kitchen waste, black bar: abattoir waste, dotted bar: swine manure

The quantity of carbohydrates that was detected in pre-pupal larvae was low (0.165 ± 0.5 mg), and comprised only 1.3% of dry body mass. There were significant differences in carbohydrate content across species for larvae fed on kitchen waste, where *C. megacephala* had the highest percentage of carbohydrates, while *L. sericata* had the lowest (Figure 4b; Table 5). There were also differences across species that fed on swine manure, with *C. chloropyga* having the lowest carbohydrate content. Larvae fed on abattoir waste had similar carbohydrate content across species. Dry mass was a significant effect when all three waste types were tested together, with carbohydrate content increasing with larval dry mass (coefficient estimate = 0.042, S.E. = 0.08).

Table 5. Summary results from a general linear model showing the effects of species, initial larval stocking rate and waste type on carbohydrate content. Only results from the analysis of all three wastes are reported, as no significant effects were detected in separate analyses of each waste type.

Waste	Effect	Mean Square	*F*	df	*p*
Kitchen	**Intercept**	**0.275**	**35.584**	**1**	**<0.001**
	Dry Mass	0.006	0.813	1	0.368
	Species	**0.042**	**5.738**	**3**	**0.001**
	Stocking rate	0.001	0.185	2	0.832
	Batch	0.014	1.902	2	0.151
	Species*Stocking rate	0.007	0.977	6	0.441
Abattoir	**Intercept**	**0.244**	**29.471**	**1**	**<0.001**
	Dry Mass	0.007	0.894	1	0.345
	Species	0.013	1.733	3	0.161
	Stocking rate	0.001	0.087	2	0.917
	Batch	0.015	1.951	2	0.144
	Species*Stocking rate	0.008	0.994	6	0.430
Swine Manure	**Intercept**	**0.260**	**50.645**	**1**	**0.001**
	Dry Mass	0.007	2.793	1	0.096
	Species	**0.006**	**2.668**	**3**	**0.049**
	Stocking rate	0.000	0.147	2	0.864
	Batch	**0.020**	**8.601**	**2**	**<0.001**
	Species*Stocking rate	0.005	2.123	6	0.052
All Waste	**Intercept**	**0.789**	**131.127**	**1**	**<0.001**
	Dry Mass	**0.026**	**4.252**	**1**	**0.040**
	Species	**0.022**	**3.722**	**3**	**0.011**
	Stocking rate	0.001	0.191	2	0.826
	Waste	0.016	2.677	2	0.069
	Species*Stocking rate	0.004	0.659	6	0.683
	Species*Waste	**0.015**	**2.522**	**6**	**0.020**
	Stocking rate*Waste	0.001	0.132	4	0.971
	Species*Stocking rate*Waste	0.009	1.417	12	0.153

Lipid content of pre-pupal larvae reared on kitchen waste was significantly affected by dry mass, species and the interaction of species and stocking rate (Table 6). Lipid content increased with an increase in dry mass (coefficient estimate = 0.036, S.E. = 0.014). Lipid content was highest at 50 larvae per 100 g of kitchen waste for *C. chloropyga*, *C. megacephala* and *L. sericata*, whereas *C. putoria* had the highest lipid content at stocking rate 100 larvae per 100 g of kitchen waste (Table B1). For *C. chloropyga* and *L. sericata* the lipid content in larvae at stocking rates of 20 and 100 larvae per 100 g of kitchen waste were similar and lower than at an initial stocking rate of 50, while lipid content was lowest for *C. megacephala* larvae at stocking rate 20 and for *C. putoria* larvae at a stocking rate of 50 larvae per 100 g of kitchen waste.

Table 6. Summary results from a general linear model showing the effects of species, initial larval stocking rate and waste type on lipid content

Waste	Effect	Mean Square	*F*-value	d.f	*p*-value
Kitchen	**Dry Mass**	**4.944**	**7.038**	**1**	**0.009**
	Species	2.213	1.328	3	0.349
	Density	2.192	2.324	2	0.214
	Batch	3.014	1.537	2	0.290
	Species*Density	**2.104**	**3.566**	**6**	**0.028**
	Error				
Abattoir	Dry Mass	0.046	0.114	1	0.736
	Species	0.622	0.903	3	0.487
	Density	**1.955**	**7.300**	**2**	**0.043**
	Batch	1.909	52.895	2	0.965
	Species*Density	0.829	0.864	6	0.547
	Error				
Swine Manure	Dry Mass	3.162	0.950	1	0.331
	Species	3.392	0.695	3	0.586
	Density	2.014	1.017	2	0.424
	Batch	18.882	5.123	2	0.123
	Species*Density	2.482	0.790	6	0.594
	Error				
All Waste	**Dry Mass**	**4.686**	**10.662**	**1**	**0.001**
	Species	0.504	0.408	3	0.751
	Density	0.548	1.130	2	0.387
	Waste	**22.610**	**47.154**	**2**	**0.001**
	Species*Density	1.058	2.964	6	0.046
	Species*Waste	1.755	2.586	6	0.095
	Density*Waste	**1.679**	**5.666**	**4**	**0.029**
	Species*Density*Waste	1.088	1.309	12	0.293
	Error				

When reared on abattoir waste, there were no significant differences in lipid content across any of the tested factors (Table 6). When all three waste types were included in the same analysis, there was a significant effect of dry mass, indicating an increase in lipid content as

dry mass increased (coefficient estimate = 0.023, S.E. = 0.007). There was also a significant species by waste interaction, an interaction of waste type and stocking rate type, and the three way interaction between species, waste and stocking rate on lipid content (Figure 4c; Table 6). At an initial stocking rate of 20, 50 and 100 larvae per 100 g of kitchen waste, pre-pupal larvae, regardless of species, had a significantly higher lipid content when fed on kitchen waste than larvae fed on swine manure at the same stocking rates (Table 6). For the species *C. chloropyga*, *C. megacephala* and *L. sericata* the highest lipid content was at 50 larvae per 100 g of kitchen waste (Table B1). In contrast, for *C. putoria* the highest lipid content was at 100 larvae per 100 g of kitchen waste, which was also the highest lipid content observed across all species, stocking rates and waste types. The lowest overall lipid content was for *C. chloropyga* at 50 larvae per 100g of swine manure. *Lucilia sericata* also had a low lipid content at 50 larvae per 100g of swine manure, whereas *C. megacephala* and *C. putoria* had lower lipid content at 100 larvae per 100 g of swine manure.

3.5 Body water content

Body water content of pre-pupal larvae was affected by wet body mass when each waste type was analysed separately. In each case, body water content increased significantly as wet mass increased (kitchen waste: coefficient estimate = 0.804, S.E. = 0.03; abattoir waste: coefficient estimate = 0.814, S.E. = 0.035; swine manure: coefficient estimate = 0.827, S.E. = 0.038). For kitchen waste and abattoir waste, species and the species by stocking rate interaction were significant effects of larval body water content, whereas for swine manure the initial stocking rate, but not species, was a significant effect. For larvae reared on swine manure, body water content was lowest at 20 and highest at 100 larvae per 100 g. When all waste types were analysed together there were significant effects of species and waste type and the interactions of species by stocking rate, species by waste, and species by stocking rate by waste on water content (Table 7). Pre-pupal larvae that were reared on swine manure had significantly higher water content than larvae fed on abattoir or kitchen waste, with the exception of *C. chloropyga* held at stocking rate 20 on abattoir waste (Figure 5; Table B2).

Table 7. Summary results from general linear models showing the effects of species, initial larval stocking rate and waste type on body water content.

Waste	Effect	Mean Square	*F*	df	*p*
Kitchen	**Intercept**	**287.866**	**20.747**	**1**	**<0.001**
	Wet Mass	**23653.523**	**1657.026**	**1**	**<0.001**
	Species	**53.838**	**3.772**	**3**	**0.011**
	Stocking rate	0.444	0.031	2	0.969
	Batch	6.352	0.445	2	0.641
	Species*Stocking rate	**73.501**	**5.149**	**6**	**<0.001**
Abattoir	**Intercept**	**102.883**	**6.044**	**1**	**<0.001**
	Wet Mass	**12740.696**	**732.543**	**1**	**<0.001**
	Species	**100.220**	**5.762**	**3**	**0.001**
	Stocking rate	22.337	1.284	2	0.279
	Batch	3.216	0.185	2	0.831
	Species*Stocking rate	**52.133**	**2.997**	**6**	**0.008**
Swine Manure	**Intercept**	**41.893**	**4.859**	**1**	**0.029**
	Wet Mass	**5926.461**	**695.678**	**1**	**<0.001**
	Species	3.414	0.401	3	0.753
	Stocking rate	**33.600**	**3.951**	**2**	**0.021**
	Batch	10.594	1.244	2	0.290
	Species*Stocking rate	12.123	1.423	6	0.207
All Waste	**Intercept**	**383.138**	**28.303**	**1**	**<0.001**
	Wet Mass	**48827.452**	**3607.018**	**1**	**<0.001**
	Species	9.706	0.717	3	0.542
	Stocking rate	6.978	0.515	2	0.597
	Waste	**403.271**	**29.791**	**2**	**<0.001**
	Species*Stocking rate	**40.276**	**2.975**	**6**	**0.007**
	Species*Waste	**80.054**	**5.914**	**6**	**<0.001**
	Stocking rate*Waste	23.292	1.721	4	0.144
	Species*Stocking rate*Waste	**53.963**	**3.986**	**12**	**<0.001**

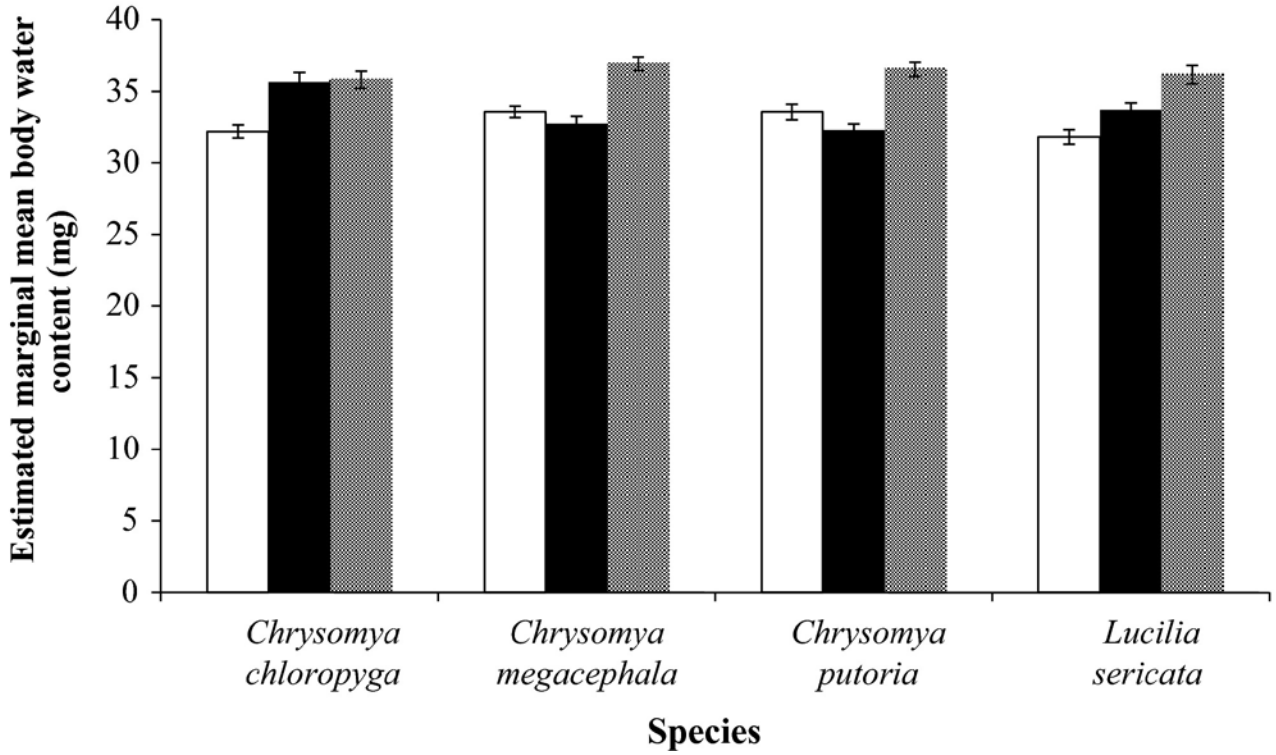

Figure 5. Water content of pre-pupal larvae of four calliphorid species fed on different waste types. Error bars indicate ±1 *SE*. Different waste types are represented by different bars. White bar: kitchen waste, black bar: abattoir waste, dotted bar: swine manure

4. Discussion

We measured how larval diet affects the development, survival and body composition of blow fly species with a view of optimising mass-rearing of blow flies using circular economic principals. Both waste type and stocking density affected larval performance, but these effects often differed between blow fly species. The relative importance of these variables depended on the trait being measured, and likely reflects the differing life histories and ecological niches of the four species studied, and their adaptability to larval substrates of varying quality.

4.1 Mass, survival and time to onset of dispersal from the substrate

On abattoir waste *C. chloropyga* had the highest mass, while *L. sericata* had the lowest mass. The relative sizes of the pre-pupal larvae feeding on this waste are a close indication of the different sizes of the adults of these flies. Adults of *L. sericata* are smaller than adult flies in

the genus *Chrysomya* (*pers. obs.*). Abattoir waste would be most similar to carrion, the natural breeding substrate for these fly species (Richards et al., 2009b). Larvae also had the greatest survival on abattoir waste. On kitchen waste, the mass and survival of the different species, excluding *C. chloropyga*, were similar. The collected kitchen waste included approximately 30% fish, which would be a highly nutritious substrate for these larvae, as *L. sericata* and *C. putoria* had greater mass when fed on kitchen waste than on abattoir waste. In the case of *C. chloropyga*, mass and survival was compromised when fed on kitchen waste and swine manure. This was associated with early onset of dispersal at low stocking rates on the same two waste types. Fly development time can vary greatly if optimal conditions are not encountered (Richards et al., 2009a; Larrain & Salas, 2008; Nguyen et al., 2013), and early dispersal by *C. chloropyga* may reflect the larvae attempting to migrate away from the substrate to find a more suitable food source. This species is a large mammal carcass specialist (Richards et al., 2009b), making swine manure and kitchen waste inappropriate for their development.

There was a general trend for higher initial stocking rate leading to a greater proportion of larvae surviving, especially in kitchen waste and swine manure. There was also minimal effect of initial stocking rate on mass of the pre-pupal larvae that fed on kitchen waste or abattoir waste, indicating that there was enough food available for the larvae in these two waste types. Larvae often aggregate when in a food source and these aggregations, also known as ‘maggot masses’, provide some advantages to the larvae, such as elevated temperatures, more efficient assimilation of the food source through mass release of digestive enzymes and reduced chances of desiccation (Anderson, 2010; Rivers et al., 2011). These advantages would lead to an increase in overall survival of larvae, so long as they do not reach the point of competition.

Larvae fed on swine manure had a low final mass and low survival and there was a strong trend towards reduced mass with increased stocking rate, except in *C. chloropyga*. This is evidence for competition between larvae for the limited nutrients available in swine manure. Other studies found that higher larval stocking rate of *M. domestica* fed on swine manure led to decreased larval mass (Moon et al., 2001; Pieterse & Gloy, 2013) and low survival (Barnard et al., 1998; Čičková et al., 2013). The two species that had the greatest mass on swine manure, *C. megacephala* and *C. putoria*, have been previously observed to be associated with manure (Laurence, 1988; Lindsay et al., 2013) and were likely more adept at extracting the necessary nutrients to survive and grow.

4.2 Larval body composition

To date, there are few studies on the nutrient content of the species investigated in this study, with the exception of *C. megacephala* (Barroso et al., 2015) and *L. sericata* (Yehuda et al., 2011; Barroso et al., 2015) (Table 1). The nutrient content detected in this study was on average 10 ± 4.9% (± 1 SD) for protein, 10.9 ± 6.2% (± 1 SD) for lipids and 1.5 ± 0.5% (± 1 SD) carbohydrates, on a dry matter basis for the different larvae. Caution is needed when comparing nutrient content from different studies as the methods used can dramatically affect results. The assays used in this study (discussed in more detail in Appendix A) focus on stored nutrients available for insect survival, growth and reproduction (Foray et al., 2012). For this reason, there is an even ratio of soluble protein to lipids because structural proteins in individual insects have not been detected by the assay. This contrasts with a heavy bias towards crude protein in other studies (Table 1), but these values may overestimate protein available to domesticated animals due to the high proportion of total insect nitrogen contained in indigestible molecules (Jonas-Levi & Martinez, 2017). The species tested in this study are all associated with carrion (Richards et al., 2009a,b) and we predicted the abattoir waste would be the most nutritious food source for these species. However, only *C. chloropyga* followed this prediction. *Chrysomya putoria*, *C. megacephala* and *L. sericata* had the highest protein content when feeding on kitchen waste. The presence of fish in the kitchen waste mixture may have represented a highly nutritious food source (Nguyen et al., 2013). Swine manure has fewer nutrients available for larvae, which resulted in reduced nutrient assimilation in flies reared on this waste.

The combination of initial stocking rate, species and waste also had an effect on the amount of lipids that were detected. *Chrysomya chloropyga*, *C. megacephala* and *L. sericata* had the highest lipid content on kitchen waste at a stocking rate of 50, whereas *C. putoria* had the highest lipid content at a stocking rate of 100 larvae per 100 g. On swine manure, low lipid content was detected at a stocking rate of 50 for *C. chloropyga* and *L. sericata* while *C. megacephala* and *C. putoria* had the lowest lipid content at stocking rate of 100. This indicates that there is no specific stocking rate that is optimal for nutrient acquisition in these species. The effect of waste type changes the lipid content present in other fly species used in bioconversion (Table 1), but the effect of stocking rate has not been studied. Overall, regardless of species, the highest lipid content was present in larvae that fed on kitchen waste. Kitchen waste would have been high in simple and complex carbohydrates due to the presence of fruit and vegetables, providing excess energy to be stored as lipids (Arrese &

Soulages, 2010). In most tested species, measured carbohydrate content of larvae was highest when fed kitchen waste. The exception to this observation was *L. sericata,* which had a higher carbohydrate content while feeding on abattoir waste. This difference across the species may be a genetic difference in how carbohydrates present in the diet are digested and assimilated (Clissold & Simpson, 2015). Carbohydrates in *L. sericata* larvae from a previous study that were fed on poultry waste were close to 10% on a dry matter basis (Yehuda et al., 2011)

Larvae that fed on swine manure had the highest body water content as well as the lowest protein and lipid content on a dry matter basis. In contrast, larvae that fed on kitchen waste had lower body water content but higher protein and lipid content than larvae that fed on swine manure. Higher nutrient content in the larval substrate leads to an increase in the presence of lipids stored in the body of the larvae (see review by Arrese & Soulages, 2010). For example, larvae of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), that are fed diets higher in protein and carbohydrates store more body lipids (Nestel et al., 2004). This increase in lipids leads to a decrease in the body water content as a percentage of body mass (Nestel et al., 2004). Our results show a similar pattern, where the highest lipid content and lowest body water content is present in larvae that fed on kitchen waste, where the highest percentage of carbohydrates for uptake and conversion to stored lipids would have been available for larvae. The higher body water content observed in larvae fed on manure also follows this pattern, as this would be the larval substrate with the lowest available nutrients, especially carbohydrates, and therefore the lowest lipid reserves were also observed in these larvae.

The relationship between body water content of larvae and diet is important for a bioconversion facility. The body water content of larvae is important for growth, where lower body water content leads to reduced growth rates and to reduced mobility and feeding (Harrison et al., 2012). There was also a strong correlation between body mass and body water content, where larger larvae tended to have greater body water content. The increased body water content for larger larvae is favourable to reduce the chances of desiccation during pupation (Rivers et al., 2013). However, increased body water content leads to lower dry matter content and ultimately reduced biomass available for the final product. Further investigation is required to determine the optimal level of body water to ensure high nutrient content in the larvae, optimal growth and survival for larvae and increased total biomass for the final product produced in a bioconversion facility.

5. Conclusion

Overall, *C. putoria* followed by *C. megacephala* were the two most versatile species that could cope with a variety of different waste types in terms of survival and final mass, and have a high protein and lipid content. However, *C. megacephala* has very low egg production, making it a less suitable species for use in a bioconversion facility (Parry et al., 2017). Kitchen waste containing fish and abattoir waste were the two best waste types for these fly species. *Chrysomya chloropyga* had high survival and greater mass on abattoir waste and was the species most suited for this waste type. However, *C. chloropyga* performed poorly on all other waste types. *Lucilia sericata* did moderately well on all three waste types, but had low survival and low mass overall and would not be recommended for use in a bioconversion facility. Increased stocking rate generally led to improved survival, although on swine manure there was also a reduction in pre-pupal larval mass. Future studies need to use higher stocking rates to determine at what stocking rate larval mass and survival are high while effective waste reduction is achieved.

Conflict of Interest Statement

No conflict of Interest

Author Contribution

- Nina Parry, Chris Weldon and Elsje Pieterse conceived the research.
- Nina Parry conducted the experiments.
- Nina Parry and Chris Weldon analysed data and conducted the statistical analyses.
- Nina Parry and Chris Weldon wrote the manuscript.
- Elsje Pieterse secured the funding.
- All authors read and approved the manuscript.

Data Availability Statement

The data that support the results of this study are available at the following link: http://hdl.handle.net/2263/69160

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