Longevity, fertility and fecundity of adult blow flies (Diptera: Calliphoridae) held at varying densities: implications for use in bioconversion of waste

Nina Jennifer Parry[1]*, Elsje Pieterse[2] and Christopher William Weldon[1]

[1] Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield 0082, Pretoria, South Africa

[2] Department of Animal Sciences, Stellenbosch University, Private Bag X1, Matieland 7602, Stellenbosch, South Africa

*Corresponding author. Email: nparry@zoology.up.ac.za, cell phone number: +27718716254

Other author email addresses:

E. Pieterse: elsjep@sun.ac.za

C. W. Weldon: cwweldon@zoology.up.ac.za

Abstract

Large numbers of flies are needed to produce the quantity of larvae required for insect bioconversion of waste. However, this "mass-rearing" may negatively affect adult survival and reproductive output. This study assessed the suitability for mass-rearing of four blow fly species, *Chrysomya chloropyga*, *C. megacephala*, *C. putoria* and *Lucilia sericata*. Flies were kept at densities of 20, 50, 100, 250, 500 and 1000 flies per 30 x 30 x 30 cm cage with an even sex ratio. Time to 50% mortality (LT50) was recorded, and the effects of density, species, and sex on LT50, fecundity and fertility were determined. Females survived longer than males across all species. There was evidence for a trade-off between survival and high fecundity in *L. sericata* and *C. chloropyga* at density 250. *Chrysomya megacephala* had low fecundity across all densities. At high densities, *C. putoria* had the lowest mortality and highest fecundity, making it the most suitable for mass-rearing.

Keywords

Mass-rearing; Bioconversion; Calliphoridae; Density; Mortality; Fecundity

Introduction

Bioconversion is a nutrient cycling process that can reduce agricultural, industrial and household organic waste, and produce value-added products. The bioconversion process can be applied to a variety of different waste types, and different organisms ranging from bacteria and yeast to insects have been used to facilitate this process (Kumar *et al.*, 2008; Madhavan *et al.*, 2012; van Huis, 2013; Čičková *et al.*, 2015). Flies (Insecta: Diptera) are often used in bioconversion due to their known association with decaying material, high fecundity, rapid generation times and ease of rearing (van Huis, 2013). Proteins and lipids recovered by bioconversion of organic waste can be fed to livestock (van Huis, 2013) or converted into biodiesel (Li *et al.*, 2011).

Large-scale conversion of waste products by insects requires that they be mass-reared. Mass-rearing of insects has been used to produce large numbers for release in sterile insect technique (SIT; Parker, 2005) and biological control programs (Sørensen *et al.*, 2012). This has included various fly species including tephritid fruit flies such as *Bactrocera tryoni*

(Froggatt) (Weldon *et al.*, 2013) and *Ceratitis capitata* (Wiedemann) (Pereira *et al.*, 2013), as well as tsetse flies (*Glossina* species; Vreysen *et al.*, 2011, Abd-Alla *et al.*, 2013) and mosquitoes (Gilles *et al.*, 2014). Insects that are kept in mass-rearing facilities should ideally not experience high mortality or suffer decreased egg production (fecundity) or egg hatch (fertility) when there is an increase in adult density within cages. However, different species of flies respond to the high density conditions of mass-rearing in different ways (Robinson, 2005), which has implications for their suitability in large-scale bioconversion facilities.

The effects of the stress associated with crowding in cages where adults are held at high density has been documented for a number of different fly species. This increased stress can lead to reduced longevity and physical injury, as well as changes in interactions between the different sexes, which then affects fecundity and fertility (Rull *et al.*, 2012). Fecundity refers to the total number of eggs that are produced by a female, whereas fertility is the number of eggs that hatch. Male mating success and therefore male reproductive success can be reduced by increased density due to changes in mating behaviour, decreased territoriality and interrupted mating with females (Gaskin *et al.*, 2002; Díaz-Fleischer *et al.*, 2009). *Drosophila melanogaster* (Meigen) show decreased levels of territoriality at high densities, leading to fewer interactions between males (Hoffmann & Cacoyianni, 1990). In *C. capitata*, male longevity is more negatively impacted by an increase in density than that of females, possibly due to increased aggression and increased behavioural costs to mate successfully (Gaskin *et al.*, 2002). Some of the changes in behavioural interactions that can occur under high density conditions have been observed in *Anastrepha ludens* (Loew), where females mate sooner in high density conditions, whereas males at lower densities mate more often (Díaz-Fleischer *et al.*, 2009). Males of *A. ludens* also require a minimal area for males to establish their territories and to reduce male–male aggressive interactions that would negatively affect their reproductive success (Díaz-Fleischer *et al.*, 2009). Egg production is one of the bottle necks in the process of mass-rearing. However, few studies have sought to maximize egg production by determining an optimal adult density for fly species that can be mass-reared in bioconversion facilities (Pastor *et al.*, 2015). This situation demands that further research be pursued to ensure high levels of fecundity and fertility while minimizing the space, cost and handling of mass-reared adults (Pastor *et al.*, 2015).

Limited research has been conducted on the use of blowflies (Calliphoridae) or flesh flies (Sarcophagidae) for mass-rearing (Čičková *et al.*, 2015; Pastor *et al.*, 2015). One exception is

the screwworm fly, *Cochliomyia hominivorax* (Coquerel), which is a major pest of cattle (Vargas-Terán *et al.*, 2005), and has been reared successfully at large scales for release in SIT programs. In *C. hominivorax*, adult density does not affect survival, fecundity or fertility (Berkebile *et al.*, 2006). However, there was an observable difference in behaviour between wild flies and those reared in the laboratory due to the selection pressure experienced during mass-rearing. These behavioural changes included an increased amount of time spent walking rather than flying and decreased competitiveness between males (Bush *et al.*, 1976). A more sedentary phenotype may have led to the laboratory reared *C. hominivorax* being less susceptible to the negative effects of increased cage density that are apparent in other mass-reared fly species.

Blow fly species have the potential to be used for bioconversion of a number of different waste products and should be studied in more detail to assess their relative utility. There are a number of organic waste streams that are not efficiently converted to biomass by the fly species currently used by most bioconversion facilities, predominantly black soldier flies, *Hermetia illucens* L., and house flies, *Musca domestica* L. (van Huis, 2013; Čičková *et al.*, 2015), and research into finding more suitable and versatile species is required. In addition, blowfly species exhibit wandering behaviour prior to pupating (Richards *et al.*, 2009a), which can be utilized in a "self-harvesting" system. This may lead to more efficient production than those using *H. illucens*, which require mechanical separation of larvae from the remaining waste (reviewed by Pastor *et al.*, 2015). Potential blowfly species include *Chrysomya megacephala* (Fabricius), *Chrysomya chloropyga* (Wiedemann), *Chrysomya putoria* (Wiedemann) and *Lucilia sericata* (Meigen) (Calliphoridae). These species have not been well studied in terms of mass-rearing with little information available on the effects of density on their life history traits. *Chrysmomya megacephala* and *C. putoria* are reported to be associated with pit latrine wastes (Laurence, 1986; Lindsay *et al.*, 2013) and may be able to successfully breakdown municipal or manure waste. Additionally, *C. megacephala* larvae have been successfully raised on restaurant waste that contained a mixture of vegetable and meat scraps (Li *et al.*, 2011). *Lucilia sericata* is a carrion-associated blowfly that is often used in laboratory experiments (Clark *et al.*, 2006; Čeřovský *et al.*, 2010; Blystone & Hansen, 2014) and is highly prolific and successful under laboratory conditions (Blystone & Hansen, 2014). *Chrysomya cholorpyga* is considered to be a large mammal carcass specialist and can

produce high numbers of eggs at a time (Richards *et al.*, 2009b). This species may be useful in the conversion of large quantities of waste.

This study assessed the suitability for mass-rearing of four species of carrion flies, specifically assessing the effects on survival, fertility and fecundity of holding adults at high densities. This is an important but often overlooked consideration for the successful establishment and continued high output of bioconversion facilities that use insects for organic waste valorisation to usable biomass. The four species tested were: *C. cholorpyga*, *C. megacephala*, *C. putoria* and *L. sericata*. These four calliphorid species are abundant in Gauteng province, South Africa, where this study was conducted (Parry *et al.*, 2016). They are also present across most of South Africa (Richards *et al.*, 2009c). *Chrysomya chloropyga* and *C. putoria* have a wide Afrotropical distribution (Picard *et al.*, 2012), while *L. sericata* and *C. megacephala* are widely distributed across most of the world (Stevens & Wall, 1996; Williams & Villet, 2006). It was hypothesized that increasing adult density would negatively affect survival of all species and both sexes, but male survival would be affected more than that of females due to the energetic demands and physical interactions associated with competition for mates. At higher densities it was also predicted that fecundity and fertility per female would be reduced due to an increase in stress and fewer or interrupted interactions between females and males.

Materials and Methods

Colony establishment

Adult carrion flies were collected from various areas around Gauteng province, South Africa, using modified Red-Top® hanging traps (Miller Methods, Ltd., Pretoria). The traps were modified by opening the base of the trap and attaching a 500 ml plastic cup with several elastic bands. Adult flies were attracted to the trap by placing a bait mixture of food-grade chicken livers and fish in the plastic cup. A square piece of white voile curtain fabric was placed over the top of the cup to stop the flies from becoming fouled in the bait. Traps were hung at a height of approximately two meters above the ground from available trees and were checked daily until the appropriate fly species and numbers were collected. Numbers of between 20 and 100 were necessary to start the initial colony, and additional flies were

caught throughout the duration of the study to maintain colony fitness and genetic variability. The species that were specifically targeted for this study were *C. chloropyga*, *C. megacephala*, *C. putoria*, and *L. sericata*.

Laboratory Rearing

The flies that were collected live from the traps were identified and kept in single species rearing cages. The cages were made from 13 L transparent plastic containers (MaxiMultiBox, Hobbylife, Istanbul, Turkey) with dimensions of 0.36 × 0.25 × 0.23 m (0.013 m^3). A 0.2 × 0.1 m^2 section was cut from the lid of the plastic container and covered with white voile curtain fabric for ventilation. One side of the cage had a circular hole cut out with a diameter of 15 cm, fitted with a 45 cm long sleeve of white voile curtain fabric for daily feeding, removal of eggs and pupae, and cleaning of the cage. The 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 approximately 25˚C in a room with a window to provide a natural day:night photocycle. Food-grade chicken liver was provided in a 150 mL plastic container (WestPack Lifestyle) as a breeding medium 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.

Effects of density on survival, fecundity and fertility

Pupae were collected from the breeding cages and placed in a separate breeding cage. Pupae were kept in an incubator (LTIE, Labcon, Gauteng, South Africa) at 25˚C until eclosion. Newly emerged flies were collected and sexed by measuring the occipital sclerite and placed into insect cages with dimensions of 0.3 × 0.3 × 0.3 m (0.028 m^3, BugDorm 43030, MegaView Science, Taichung, Taiwan). Flies were kept at different densities with an even sex ratio (Table 1). Each density was repeated five times for each species. The density of 1000 flies per cage was as high as or higher than the high densities that are found within bioconversion facilities based on the available cage surface area and volume per fly. Reported values from bioconversion facilities are between 2.5 cm^2 and 10 cm^2 internal cage surface area per fly or a volume of between 25 cm^3 and 200 cm^3 per fly (Charlton *et al.*, 2015).Each cage was checked daily for fly mortality. Sex of fatalities was recorded. From 8 until 32 days after adult emergence, a Petri dish containing 30 g of liver was placed in each cage every second day. Within 36 hours, the liver was removed and assessed for eggs laid in order to determine female fecundity. If eggs were present, they were carefully collected, weighed to

determine the approximate number of eggs, and then placed on fresh liver where they were allowed to hatch. The approximate mass of an egg per species is 0.101 ± 0.024 mg for *C. chloropyga*, 0.128 ± 0.021 mg for *C. megacephala*, 0.124 ±0.023 mg for *C. putoria* and 0.119 ± 0.031 mg for *L. sericata*. The number of eggs that did not hatch into first instar larvae were counted the next day to determine egg viability. This was used to assess fertility of the females present at different densities. Recording of mortality and assessment of fecundity and fertility continued until 45 days after adult emergence or until up to 80% of females had died.

Table 1. The cage surface area and cage volume that is available per fly for each initial fly density.

Initial Fly Density	Surface area (cm^2)/fly	Volume (cm^3)/fly
20	270	1350
50	108	540
100	54	270
250	21.6	108
500	10.8	54
1000	5.4	27

Data analysis

The time elapsed until 50% of females and males were dead (LT50) was determined for each replicate cage of each species and density. A general linear model was used to determine the effect of density, species, sex, and their interactions on LT50. Generalized linear models were used to analyse the effect of density, species and time, and their interactions, on the number of eggs per female that were laid over time. The time (in days) until peak production and the number of eggs that were laid per female on the day of peak egg production were also analyzed in relation to density, species, and their interaction. These measures of fecundity are informative for the harvesting of eggs in bioconversion facilities. Step-wise deletion of the least significant terms was used to minimise the model. Post-hoc multiple comparisons were performed using Fisher's least significant difference (LSD) tests. IBM SPSS Statistics was used to perform these analyses. A logistic regression with a quasibinomial distribution was applied to the total number of eggs produced combined with the total number of hatched eggs, both accounting for the initial density of females (included as a covariate in the model), in order to determine the effect of density and species on egg fertility. A quasibinomial distribution was applied due to over dispersion of the data when using a binomial distribution. In all cases, the minimal adequate model was obtained by applying a model simplification procedure based on Akaike's information criterion (Crawley, 2007).

Differences between groups were identified by inspecting model coefficients. These analyses were performed using RStudio version 0.99.903 running R version 3.3.1 (R Development Core Team, 2016).

Results

Survival

Survival for each species at each density was determined by looking at the time in days until 50% of females and males had died (LT50) for each cage. LT50 differed significantly between females and males (Figure 1; $F_{1, 217} = 24.669$, $p < 0.001$), with females reaching 50% mortality later than males. Significant differences in LT50 were also found between species ($F_{3, 217} = 17.871$, $p < 0.001$), between densities ($F_{5, 217} = 9.261$, $p < 0.001$), and within species by density interactions ($F_{15, 217} = 4.763$, $p < 0.001$).

Due to differences in the effect of density within different species, there was no clear trend for the overall effect of increasing density on LT50 (Figure 1). At density 250, *C. chloropyga* and *L. sericata* were significantly different from all other species, with LT50 reached before all other species and densities. LT50 was reached last by *C. putoria* females at density 250 and 100. *Chrysomya megacephala* and *C. putoria* were not significantly different from each other across all densities and followed a trend of high survival at densities 50, 100 and 250 with a decrease in survival at density 500. Across all four species there was an increase in LT50 from density 500 to density 1000. At density 50 or 1000, the LT50 of the species did not differ from each another. *Lucilia sericata* and *C. chloropyga* were only significantly different from each other at density 100, where LT50 was reached earlier by *C. chloropyga* than by *L. sericata*. These two species had a similar response to increasing density in the cages, with a decrease in LT50 from density 50 to density 250 and from there an increase in LT50 from density 250 to 1000.

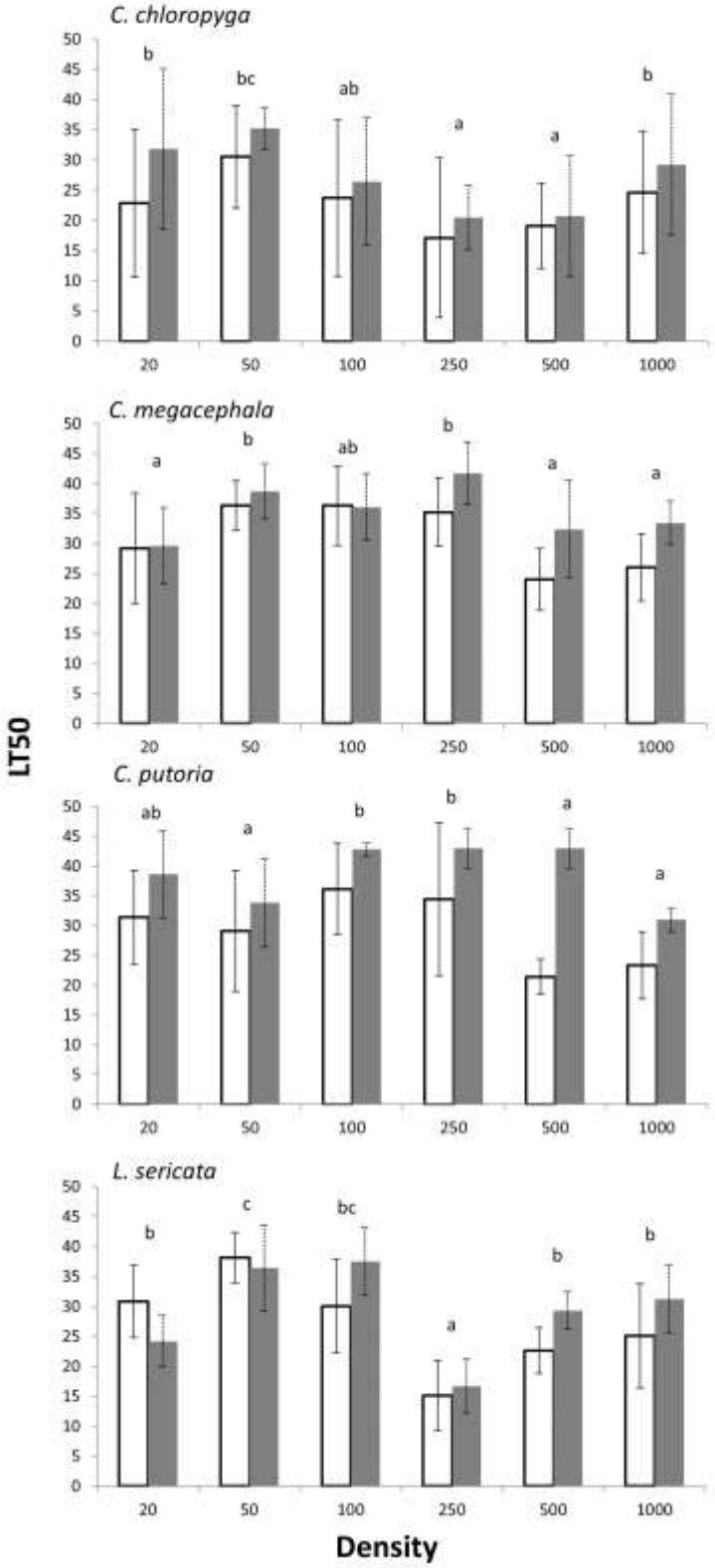

Figure 1. Time until 50% mortality (LT50) of females and males of four calliphorid species held at different initial densities. Significant differences between different densities within a species are indicated by letters. Solid white bar = male, dotted bar = female. Significant differences between different densities within a species are indicated by letters with homogenous groups sharing the same letters.

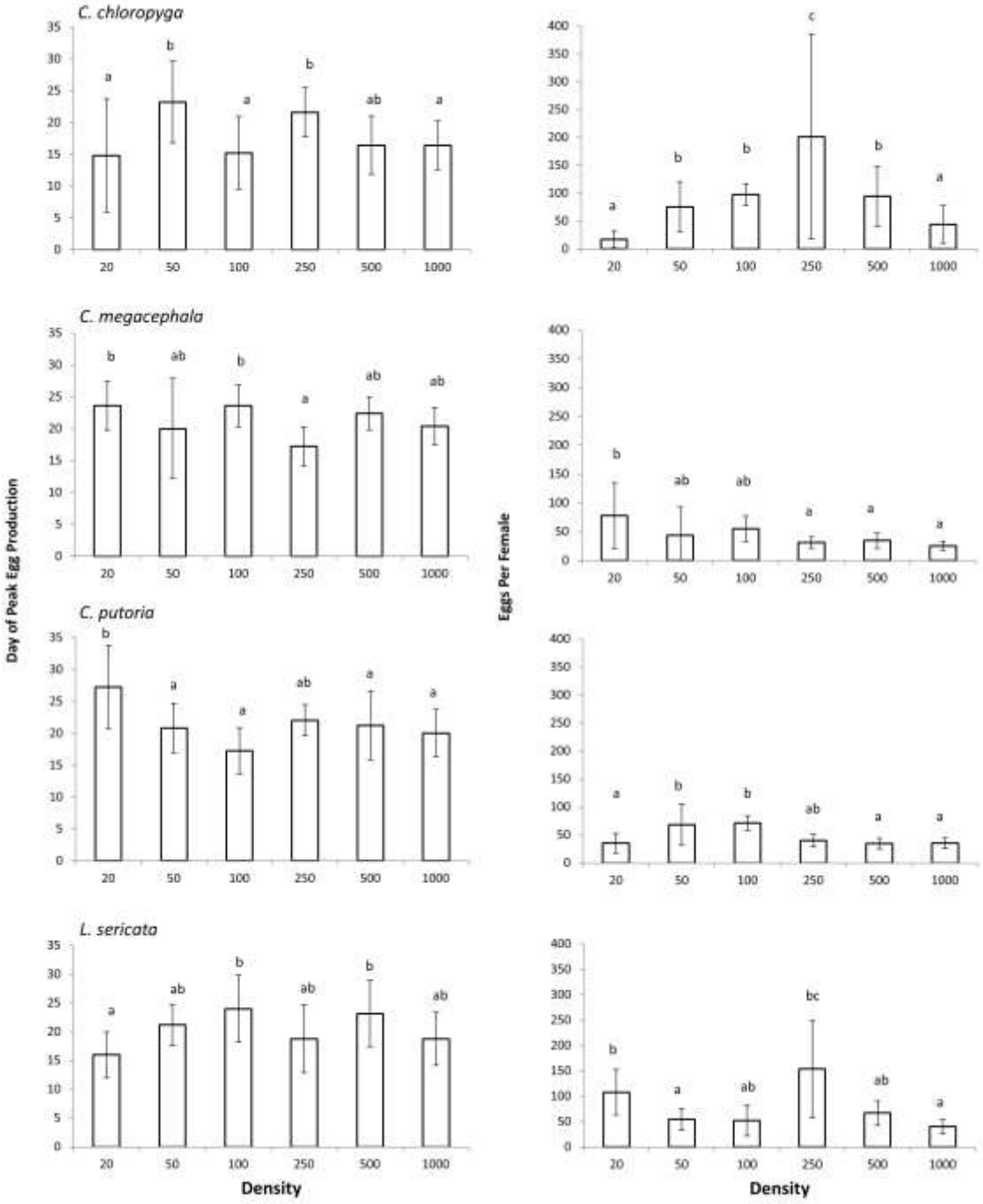

Figure 2. Day of peak egg production for four calliphorid species held at different densities and number of eggs produced per female on day of peak egg production. Significant differences between different densities within a species are indicated by letters.

Fecundity

Time elapsed (in days) until peak egg production (Figure 2) was significantly different among species (GLZ: $\chi^2 = 11.218$, df = 3, p = 0.011) and was also affected by the interaction of species and density (GLZ: $\chi^2 = 37.876$, df = 15, $p < 0.001$). The main effect of density was not significant (GLZ: $\chi^2 = 3.714$, df = 5, p = 0.591), indicating the absence of a clear trend of the effect of increasing density on the day of peak egg production across all four species. The earliest day of peak egg production among the four species was achieved by *C. chloropyga* at density 20 while the latest day of peak egg production was achieved by *C. putoria* at density of 20. For *C. putoria* as density increased from density 20 the time to day of peak egg production markedly decreased until density 100. Thereafter, there was no effect of density on day of peak egg production for *C. putoria*. *Lucilia sericata* showed a different pattern from *C. putoria* where the density 20 reached peak egg production first followed by an increase in the time until peak egg production as density increased, with the exception of density 250 and 1000. These two densities were not significantly different from density 20. There were no significant differences of day of peak egg production across different species at densities 50, 250 and 1000.

The number of eggs produced per female on the day of peak egg production was significantly affected by species (GLZ: $\chi^2 = 17.07$, df = 3 $p < 0.001$), density (GLZ: $\chi^2 = 20.64$, df = 5, $p < 0.001$), and the interaction of species and density (GLZ: $\chi^2 = 41.56$, df = 15, $p < 0.001$). The number of eggs produced per female on day of peak egg production did not vary with density among *C. megacephala* and *C. putoria*. For *L. sericata* and *C. chloropyga* the highest number of eggs produced per female was at density 250. As density increased or decreased from density 250, there was a decrease in the number of eggs produced per female for both species, with the exception of density 20 for *L. sericata*.

The number of eggs produced per female per day (Figure 3) was significantly affected by species (GLZ: $\chi^2 = 89.34$, df = 3 $p < 0.0005$), density (GLZ: $\chi^2 = 52.00$, df = 5, $p < 0.0005$), time (in days) (GLZ: $\chi^2 = 120.474$, df = 13, $p < 0.0005$), and the interaction of species and density (GLZ: $\chi^2 = 100.62$, df = 15, $p < 0.0005$). At density of 20, *L. sericata* was significantly different from all other species and all other densities within *L. sericata*, with a higher overall average number of eggs produced per female every second day between day eight and day 34. At density 250, *L. sericata* and *C. chloropyga* were significantly different from all other species and produced more eggs per day than *C. putoria* and *C. megacephala*.

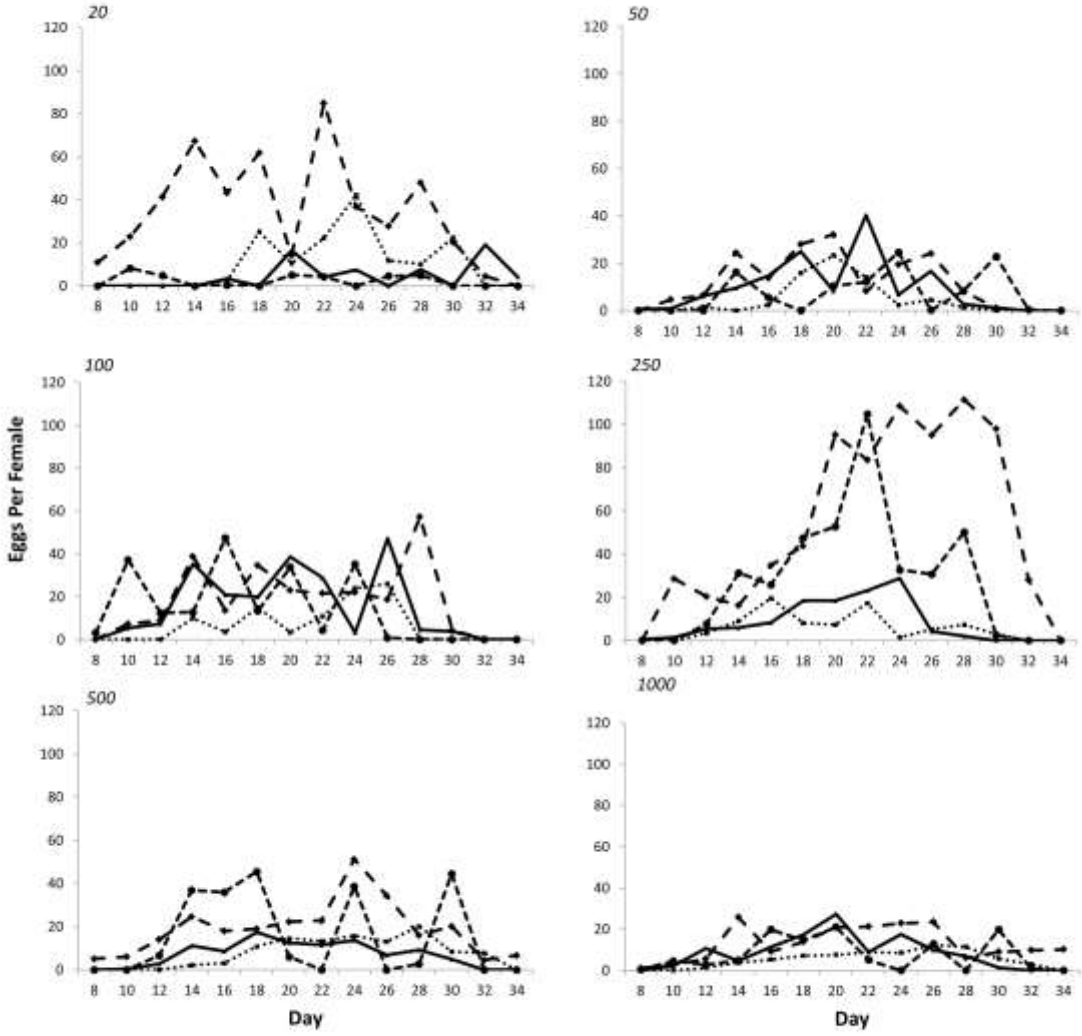

Figure 3. Average number of eggs produced per female per day across six different densities for four calliphorid species. Line style represents different species: short dash with circle markers = *C. chloropyga*, dots with square markers = *C. megacephala*, solid = *C. putoria*, long dash with diamond markers = *L. sericata*.

At density 250 *C. putoria* and *C. megacephala* were not significantly different from each other. At density 500, *L. sericata* was significantly different from *C. megacephala* and *C. putoria* with an overall higher egg production per female per day. There were no significant differences across species for density 50, 100 or 1000. *Lucilia sericata* at density 250 was significantly different from all other densities, with the highest average egg production per

female per day. *Chrysomya chloropyga* was also significantly different from other species and densities at density 250, with a high average number of eggs produced per female per day. *C. chloropyga* at density of 20 had the lowest total number of eggs produced per female across all species.

Fertility

Effects of species and density on fertility were determined by relating the total number of eggs produced over time for each species to the total number of eggs that hatched and the initial density of females (Figure 4). There were significant differences between the species (Logistic Regression: $\chi^2 = 14.72$, df = 3, $p < 0.0005$), different densities (Logistic Regression: $\chi^2 = 32.59$, df = 5, $p < 0.0005$), and within species by density interactions (Logistic Regression: $\chi^2 = 34.16$, df = 15, $p < 0.0005$). Overall there were no strong effects of density on the overall fertility of eggs, with the exception of *C. chloropyga* at density of 20 which had the lowest fertility across all species and densities. *Chrysomya chloropyga* and *C. putoria* were significantly different from all other species at density 20, with *C. putoria* also exhibiting low fertility at density 20, while *C. megacephala* and *L. sericata* were not significantly different from each other at this density. At density 100, *C. putoria* and *C. chloropyga* were similar to each other and *C. megacephala* and *L. sericata* were not significantly different from each other. Fertility of *C. chloropyga* was significantly higher than *L. sericata* at density 500. There were no significant differences in fertility across species at densities of 50, 250 and 1000. There were no significant differences in fertility across the different densities for *C. megacephala*.

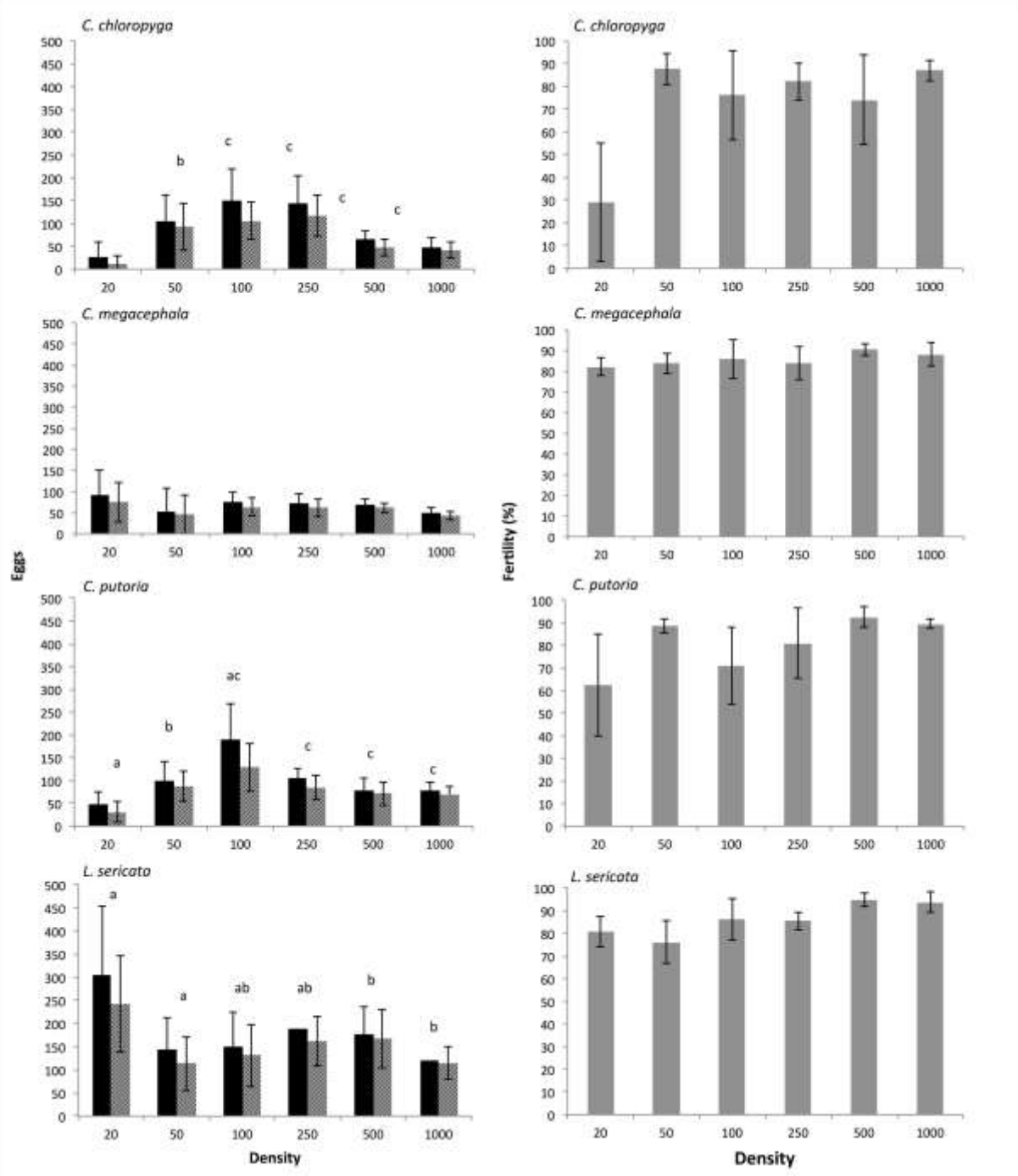

Figure 4. Average total number of eggs produced over time, and average total number of hatched eggs divided by the initial female density for each density for four calliphorid species. Fertility was calculated by dividing total number of hatched eggs by total number of eggs produced over time, presented as a percentage. Significant differences between different densities within a species are indicated by letters. Solid white bar = average total number of eggs produced standard deviation, dotted bar = average total number of hatched eggs with standard deviation.

Discussion

Survival

Survival was strongly affected by an increase in density, but not in a linear manner. The effect of density is complex across the four tested species and it may be that the stress associated with crowding is not the only factor that influences survival. All four species had a similar response to the increase in density from 500 to 1000, with an overall but slight increase in survival and no significant differences across species. This may be an example of behavioural flexibility in response to the high density and reduced space to move around. At such a high density the flies present in these cages may have responded to the reduced space by flying less and walking more, reducing the stress and potential physical damage associated with increased crowding (Bush *et al.*, 1976), making the response to survival similar for both densities.

Both females and males experienced similar responses to density. However, males may have expended more energy or experienced more competition than females, leading to overall higher mortality in males. This trend in survival between females and males has previously been recorded in *L. sericata* (Moe *et al.*, 2002; Rueda *et al.*, 2010), but no mechanism was proposed and little is known about the reproductive behaviour or the potential effects of mating interactions on females and males in carrion-associated flies in the family Calliphoridae. In the tephritid fly, *C. capitata*, males exhibited higher mortality than females in response to increased density due to more male-male interactions and courting of females (Gaskin *et al.*, 2002; Papadopoulos *et al.*, 2010). Adult male *C. homnivorax* experience higher mortality than females under a number of different experimental conditions, including different initial densities, diets and temperatures (Berkebile *et al.*, 2006).

Fecundity

There was a reduction in fecundity at low densities for *C. chloropyga* and *C. putoria*. *Chrysomya chloropyga* is a large mammal carcass specialist, which congregates in large numbers around a suitably sized carcass (*pers. obs.*). It may be that at very low densities such as density 20 there are too few interactions between females and males to stimulate mating and oviposition, a response to low densities that has been observed in the field for a number of different insects (Rhainds, 2010). *Chrysomya putoria* at low densities took a long time to reach peak egg production which may indicate that more time was required for adult flies at

low densities to mate enough for eggs to be produced than at higher densities. As density increased the time to day of peak egg production was reached earlier, indicating that mating was occurring sooner, leading to females laying their eggs sooner. This also led to an increase in eggs produced per female until density 250. Thereafter there was no effect of density on the number of eggs produced per female over time or time to day of peak egg production, indicating that at higher densities there were enough opportunities for females to mate to consistently produce eggs (Ridley, 1988).

In comparison with *C. chloropyga* and *C. putoria*¸ *L. sericata* reached peak egg production and highest number of eggs produced per female at the lowest density. Time until peak egg production increased and the number of eggs produced per female tended to decline in response to increasing density. In addition, *L. sericata* exhibited the highest production of eggs across all densities in comparison with other species. Egg production of *L. sericata* in this study corresponds well with the number of eggs produced per female in other studies (Pitts &Wall 2004; Davies 2006; Rueda *et al.*, 2010). The high egg production per female observed in *L. sericata* at density 20 and the early day of peak egg production may be due to a reduction in competition between females for oviposition sites (Moe *et al.*, 2002). In a study performed by Delves and Browne (1989), it was found that new colonies of *Lucilia cuprina* (Wiedemann), a closely related species to *L. sericata*, that had not yet adapted to lab conditions had the highest egg production at very low densities. The reduction in egg production at higher densities was due to an inhibitory effect on oviposition behaviour as a result of increased competition for oviposition sites. This effect disappeared as the colony was maintained over a number of generations and the flies became more adapted to the lab environment (Delves & Browne, 1989).

Although all species at all densities had *ad libitum* access to sugar, milk powder and water, the size of the dish and the amount of liver provided did not increase with increasing density. This may have led to an increase in competition between females for sites to oviposit as density increased, leading to reduced fecundity of females with increasing density. This pattern is reflected from density 250 to density 1000 across all species except *C. megacephala*. *Chrysomya megacephala* had a much lower egg production than the other species so it is likely that females did not encounter the strong competition for a limited resource experienced by the other species.

The highest fecundity over the entire period of egg production was recorded in *L. sericata* and *C. chloropyga* at density 250. For *C. chloropyga* and *L. sericata* this was the density where the lowest survival was observed and therefore it would indicate that a higher fecundity leads to a decrease in survival over time. It was also observed for these two species that where there was lower egg production there was higher survival, even with increasing density. This trade-off between fecundity and longevity is well documented across a number of other fly species such as *A. ludens* (Carey *et al.*, 2008), *B. tryoni* (Fanson *et al.*, 2012) and *D. melanogaster* (Gasser *et al.*, 2000). This trade-off has been linked to nutrition, where a high protein to carbohydrate ratio leads to reduced longevity (Carey *et al.*, 2008; Fanson *et al.*, 2012). However, there was no observed trade-off in survival and egg production in *C. putoria* or in *C. megacephala*. The highest egg production for *C. putoria* was at density 100. It may be that a trade-off between survival and egg production was not observed under the experimental conditions of this study for this species. Egg production did not change at all across different densities for *C. megacephala*, remaining low across all densities in comparison to the egg production of the other species.

The average number of eggs produced per female in a cage decreased over time across all densities and species. Although these flies have the potential to survive past 45 days, the females produce very low numbers of eggs after day 30 across all densities and species. This is probably due to a combination of senescence and a decrease in the density of males available for further mating.

Fertility

There was not as strong an effect of increased density on fertility. There was an increase in fertility in response to an increase in density for all species except *C. megacephala*, despite the decreased fecundity at higher densities. The increase in fertility in comparison to fecundity is likely due to an increase in opportunities for repeat mating and an increase in competition between males for females, leading to increased duration of copulation or more sperm transfer during mating to females (Kelly & Jennions, 2011), which would lead to higher fertility.

There was a reduction in fertility at low densities for *C. chloropyga* and *C. putoria*. This may be due to sperm depletion through reduced opportunities for remating or shorter copulations in the absence of competition between males for these two species. These hypotheses require further study with regards to these two species. When females are unable to remate this leads

to a reduction in egg fertility (Abraham *et al.*, 2011). The lowest fertility was recorded for *C. chloropyga* at density 20. *Chrysomya megacephala* did not show any strong differences in fertility across the different densities. However, due to the observed low egg production in this species, there may not have been an effect of reduced mating opportunities as the females did not lay large numbers of eggs.

Implications for mass-rearing

Within a mass-rearing facility the most important factors for efficient production of larvae are high egg production, low mortality and a large number of fertile adults in as little space as possible. The species that had the lowest mortality while maintaining high egg production at a high density was *C. putoria*, which makes it the best candidate for mass-rearing that was assessed in this study. Although *L. sericata* had a much higher egg production than any of the other species, their optimal density was low and not ideal for efficient use of space within a facility for mass-rearing. *Chrysomya chloropyga* also produced high numbers of eggs, but fecundity and fertility were more density dependent, and survival and egg production were low at higher densities. Egg production and fertility of *Chrysomya megacephala* did not change in response to density, but this species had low fecundity, making it less suitable for the production of large numbers of larvae for bioconversion of waste when space is limited. None of these fly species should be kept for longer than 32 days as they are no longer producing large numbers of eggs past this point. After day 32, flies should be replaced with a new cohort in order to maintain a consistent supply of eggs and larvae.

Selective pressures inside a mass-rearing facility affect insect performance through adaptation to the mass-rearing environment, including indirectly from inbreeding depression and by direct effects such as crowding and artificial diets (Sørensen *et al.*, 2012). Under natural conditions, flies are found clumped around resources such as host plants or food sources among regions of substantially lower density (Lance & McInnis 2005). These areas of higher density facilitate opportunities for breeding. However, keeping flies in permanent high density conditions can lead to an increase in potential physical damage due to crowding, competition and aggression which ultimately leads to an increase in mortality (Gaskin *et al.*, 2002). This high degree of mortality is often observed at the start of the domestication process, as individuals that are better adapted to the mass-rearing environment are selected for and survive better (Itô & Yamamura, 2005). For mass-rearing of insects for bioconversion, selection leads to high productivity, specifically high egg production. Once

flies have been collected from the wild and are bred in cages with high densities, there is also selection for flies that are less active and less easy to startle (Weldon *et al.*, 2010), as well as strains that are affected less by stress, leading to decreased rates of mortality in response to high densities. This intentional selection pressure can increase the performance of flies under laboratory conditions, leading to reduction in the effects of increased density (Miyatake, 1998).

The results of this study indicate that *C. putoria* is the most suited to the high density conditions experienced in a bioconversion facility and will adapt rapidly and efficiently to mass-rearing conditions. However, the flies that were used in this study were not completely laboratory-adapted, although they had been kept for a number of generations under laboratory conditions to complete this study. Due to the high potential for laboratory adaptation in these fly species it is necessary to continue with further studies to track changes in survival, fertility and fecundity over successive generations in the laboratory to investigate the potential beneficial effects of laboratory adaptation. Laboratory adaptation may benefit their survival at high density, and could lead to higher fecundity and decreased mortality at higher densities, especially in *L. sericata*, due to selection of flies that are tolerant of high density conditions. Furthermore, the suitability of the different species for the degradation of particular waste materials and conversion into usable biomass must also be determined to assess their suitability for use in bioconversion facilities in the future.

Acknowledgements

Jian Du Plooy and Johan Saayman are thanked for their assistance with the maintenance of fly cultures and experiments. Kevin Malod is thanked for reviewing an earlier version of the manuscript. N. J. P. is an MSc student funded by the South African National Research Foundation (SFH150718127604) and AgriProtein Technologies. Research was funded by a University of Pretoria Research Development Programme grant awarded to C.W.W..

References

Abd-Alla, A. M., Bergoin, M., Parker, A. G., Maniania, N. K., Vlak, J. M., Bourtzis, K., Boucias, D. G. & Aksoy, S. (2013) Improving sterile insect technique (SIT) for tsetse flies through research on their symbionts and pathogens. Journal of Invertebrate Pathology **112**, S2-S10.

Abraham, S., Goane, L., Rull, J., Cladera, J., Willink, E. & Vera, M.T. (2011) Multiple mating in *Anastrepha fraterculus* females and its relationship with fecundity and fertility. Entomologia Experimentalis et Applicata **141,** 15-24.

Berkebile, D.R., Sagel, A., Skoda, S.R. & Foster, J.E. (2006) Laboratory environment effects on the reproduction and mortality of adult screwworm (Diptera: Calliphoridae). Neotropical Entomology **35,** 781-786.

Blystone, A.M. & Hansen, K.M. (2014) A comparison of common diets for the continuous culture of adult *Lucilia sericata* (Diptera: Calliphoridae) for forensic and medical entomological applications. Journal of Medical Entomology **51,** 1296-1303.

Bush, G.L., Neck, R.W. & Kitto, G.B. (1976) Screwworm eradication: inadvertent selection for noncompetitive ecotypes during mass rearing. Science **193,** 491-493.

Carey, J.R., Harshman, L.G., Liedo, P., Müller, H.G., Wang, J.L. & Zhang, Z. (2008) Longevity–fertility trade-offs in the tephritid fruit fly, *Anastrepha ludens*, across dietary-restriction gradients. Aging Cell **7,** 470-477.

Čeřovský, V., Žďárek, J., Fučík, V., Monincová, L., Voburka, Z., & Bém, R. (2010) Lucifensin, the long-sought antimicrobial factor of medicinal maggots of the blowfly *Lucilia sericata*. Cellular and Molecular Life Sciences **67,** 455-466.

Charlton, A. J., Dickinson, M., Wakefield, M. E., Fitches, E., Kenis, M., Han, R., Zhu, F., Kone, N., Grant, M., Devic, E., Bruggeman, G., Prior R. & Smith, R. (2015) Exploring the chemical safety of fly larvae as a source of protein for animal feed. Journal of Insects as Food and Feed **7**-16.

Čičková, H., Newton, G. L., Lacy, R. C. & Kozánek, M. 2015. The use of fly larvae for organic waste treatment. Waste Management **35,** 68-80.

C**lark, K., Evans, L. & Wall, R.** (2006) Growth rates of the blowfly, *Lucilia sericata*, on different body tissues. Forensic Science International **156,** 145-149.

Crawley, M.J. 2007. Statistical modelling pp. 323-386 in The R Book.

Davies, L. (2006) Lifetime reproductive output of *Calliphora vicina* and *Lucilia sericata* in outdoor caged and field populations; flight vs. egg production?. Medical and Veterinary Entomology **20,** 453-458.

Delves, R.I. & Browne, L.B. (1989) Changes in reproductive performance of the Australian sheep blowfly, *Lucilia cuprina* (Wied.) (Dipt., Calliphoridae) following laboratory colonization. Journal of Applied Entomology **107,** 228-237.

Díaz-Fleischer, F., Arredondo, J. & Aluja, M. (2009) Enriching early adult environment affects the copulation behaviour of a tephritid fly. Journal of Experimental Biology **212,** 2120-2127.

Fanson, B.G., Fanson, K.V. & Taylor, P.W. (2012) Cost of reproduction in the Queensland fruit fly: Y-model versus lethal protein hypothesis. Proceedings of the Royal Society of London B: Biological Sciences **279,** 4893-4900.

Gaskin, T., Futerman, P. & Chapman, T. (2002) Increased density and male–male interactions reduce male longevity in the medfly, *Ceratitis capitata*. Animal Behaviour **63,** 121-129.

Gasser, M., Kaiser, M., Berrigan, D. & Stearns, S.C. (2000) Life-history correlates of evolution under high and low adult mortality. Evolution **54,** 1260-1272.

Gilles, J.R., Schetelig, M.F., Scolari, F., Marec, F., Capurro, M.L., Franz, G. & Bourtzis, K. (2014) Towards mosquito sterile insect technique programmes: exploring genetic, molecular, mechanical and behavioural methods of sex separation in mosquitoes. Acta Tropica **132,** S178-S187.

Hoffmann, A.A. & Cacoyianni, Z. (1990) Territoriality in *Drosophila melanogaster* as a conditional strategy. Animal Behaviour **40,** 526–537.

Itô, Y. & Yamamura, K. (2005) Role of population and behavioural ecology in sterile insect technique pp. 117 – 208 in Dyck *et al.*, (Ed.) Sterile Insect Technique, Austria, IAEA.

Kelly, C.D. & Jennions, M.D. (2011) Sexual selection and sperm quantity: meta- analyses of strategic ejaculation. Biological Reviews **86,** 863-884.

Kumar, R., Singh, S., & Singh, O.V. (2008) Bioconversion of lignocellulosic biomass: biochemical and molecular perspectives. Journal of Industrial Microbiology and Biotechnology **35,** 377-391.

Lance, D.R. & Mcinnis, D.O. (2005) Biological basis of the sterile insect technique pp. 69 – 94 in Dyck *et al.*,, (Ed.) Sterile Insect Technique, Austria, IAEA.

Laurence, B.R. (1986) Old world blowflies in the New World. Parasitology Today **2,** 77-79.

Li, Q., Zheng, L., Qiu, N., Cai, H., Tomberlin, J.K. & Yu, Z. (2011) Bioconversion of dairy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production. Waste Management **31,** 1316-1320.

Lindsay, T.C., Jawara, M., D'alessandro, U., Pinder, M. & Lindsay, S.W. (2013) Preliminary studies developing methods for the control of *Chrysomya putoria*, the African latrine fly, in pit latrines in The Gambia. Tropical Medicine & International Health **18,** 159-165.

Madhavan, A., Srivastava, A., Kondo, A. & Bisaria, V. S. (2012) Bioconversion of lignocellulose-derived sugars to ethanol by engineered *Saccharomyces cerevisiae*. Critical Reviews in Biotechnology **32,** 22-48.

Miyatake, T. (1998) Genetic changes of life history and behavioural traits during mass rearing in the melon fly, *Bactrocera cucurbitae* (Diptera: Tephritidae). Researches on Population Ecology **40,** 301-310.

Moe, S.J., Stenseth, N.C. & Smith, R.H. (2002) Density dependence in blowfly populations: Experimental evaluation of non-parametric time-series modelling. Oikos **98,** 523-533.

Papadopoulos, N. T., Liedo, P., Müller, H. G., Wang, J. L., Molleman, F. & Carey, J. R. (2010) Cost of reproduction in male medflies: the primacy of sexual courting in extreme longevity reduction. Journal of Insect Physiology **56,** 283-287.

Parker, A.G. (2005) Mass rearing for sterile insect release pp. 209 – 232 in Dyck *et al.*,, (Ed.) Sterile Insect Technique, Austria, IAEA.

Parry, N.J., Mansell, M.W. & Weldon, C.W. (2016). Seasonal, locality, and habitat variation in assemblages of carrion-associated Diptera in Gauteng Province, South Africa. Journal of Medical Entomology **53,** 1322-1329.

Pastor, B., Velasquez, Y., Gobbi, P. & Rojo, S. (2015) Conversion of organic wastes into fly larval biomass: bottlenecks and challenges. Journal of Insects as Food and Feed **1,** 179-193.

Pereira, R., Yuval, B., Liedo, P., Teal, P.E.A., Shelly, T.E., McInnis, D.O. & Hendrichs, J. (2013) Improving sterile male performance in support of programmes integrating the sterile insect technique against fruit flies. Journal of Applied Entomology **137,** 178-190.

Picard, C.J., Villet, M.H. & Wells, J.D. (2012) Amplified fragment length polymorphism confirms reciprocal monophyly in *Chrysomya putoria* and *Chrysomya chloropyga*: a correction of reported shared mtDNA haplotypes. Medical and Veterinary Entomology **26,** 116-119.

Pitts, K.M. & Wall, R. (2004) Adult mortality and oviposition rates in field and captive populations of the blowfly *Lucilia sericata*. Ecological Entomology **29,** 727-734.

Rhainds, M. (2010) Female mating failures in insects. Entomologia Experimentalis et Applicata **136,** 211-226.

Richards, C. S., Crous, K. L. & Villet, M. H. (2009a) Models of development for blowfly sister species *Chrysomya chloropyga* and *Chrysomya putoria*. Medical and Veterinary Entomology **23,** 56-61.

Richards, C.S., Price, B.W. & Villet, M.H. (2009b) Thermal ecophysiology of seven carrion-feeding blowflies in Southern Africa. Entomologia Experimentalis et Applicata **131,** 11-19.

Richards, C.S., Williams, K.A. & Villet, M.H. (2009c). Predicting geographic distribution of seven forensically significant blowfly species (Diptera: Calliphoridae) in South Africa. African Entomology **17,** 170-182.

Ridley, M. (1988) Mating frequency and fecundity in insects. Biological Reviews **63,** 509-549.

Robinson, A. S. (2005) Genetic basis of the sterile insect technique pp. 96 – 110 in Dyck *et al.,*, (Ed.) Sterile Insect Technique, Austria, IAEA.

Rueda, L. C., Ortega, L. G., Segura, N. A., Acero, V. M. & Bello, F. (2010) *Lucilia sericata* strain from Colombia: Experimental colonization, life tables and evaluation of two artificial diets of the blowfy *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), Bogotá, Colombia Strain. Biological Research **43,** 197-203.

Rull, J., Birke, A., Ortega, R., Montoya, P. & López, L. (2012) Quantity and safety vs. quality and performance: conflicting interests during mass rearing and transport affect the efficiency of sterile insect technique programs. Entomologia Experimentalis et Applicata **142,** 78-86.

Sørensen, J.G., Addison, M.F. & Terblanche, J.S. (2012) Mass-rearing of insects for pest management: Challenges, synergies and advances from evolutionary physiology. Crop Protection **38,** 87-94.

Stevens, J. & Wall, R. (1996) Species, sub-species and hybrid populations of the blowflies *Lucilia cuprina* and *Lucilia sericata* (Diptera: Calliphoridae). Proceedings of the Royal Society of London B: Biological Sciences **263,** 1335-1341.

Van Huis, A. (2013) Potential of insects as food and feed in assuring food security. Annual Review of Entomology **58,** 563-583.

Vargas-Terán, M., Hofmann, H.C. & Tweddle, N.E. (2005) Impact of screwworm eradication programmes using the sterile insect technique pp. 629-650 in Sterile Insect Technique. Springer, Netherlands

Vreysen, M.J., Saleh, K.M., Lancelot & R., Bouyer, J. (2011) Factory tsetse flies must behave like wild flies: a prerequisite for the sterile insect technique. PLoS Neglected Tropical Diseases **5,** e907.

Williams, K.A. & Villet, M.H. (2006). A new and earlier record of *Chrysoma megacephala* in South Africa, with notes on another exotic species, *Calliphora vicina* (Diptera: Calliphoridae). African Invertebrates **47,** 347.

Weldon, C.W., Prenter, J., & Taylor, P.W. (2010) Activity patterns of Queensland fruit flies (*Bactrocera tryoni*) are affected by both mass- rearing and sterilization. Physiological Entomology **35,** 148-153.

Weldon, C.W., Yap, S. & Taylor, P.W. (2013). Desiccation resistance of wild and mass-reared *Bactrocera tryoni* (Diptera: Tephritidae). Bulletin of Entomological Research **103,** 690-699.