A Stage-structured Individual-based Model for Ecological and Evolutionary Dynamics of *Drosophila melanogaster* Populations Adapted for Larval Crowding

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A dissertation submitted for the partial fulfilment of

BS-MS dual degree in Science

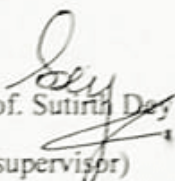## Indian Institute of Science Education and Research, Mohali

April 2020

Certificate of Examination

This is to certify that the dissertation titled "**A Stage-structured Individual-based Model for Ecological and Evolutionary Dynamics of *Drosophila melanogaster* Populations Adapted for Larval Crowding**" submitted by Mr. Sayyed Imran Rashid (Reg. No. MS15139) for the partial fulfilment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

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Dated: May 2, 2020

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. N. G. Prasad at the Indian Institute of Science Education and Research Mohali and Prof. Sutirth Dey at the Indian Institute of Science Education and Research Pune.
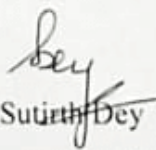
This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

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In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. N. G. Prasad

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Acknowledgements

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Abstract

Larval crowding has been shown to influence evolution of higher competitive ability in *Drosophila*. However the effect of ecological dynamics in larval crowding environment on the evolution of larval traits is relatively less explored.

Here I describe a stage-structured individual-based model to investigate the evolution of larval traits in *Drosophila melanogaster* populations adapted for various larval crowding conditions. The model also describes the ecological dynamics during larval feeding by simulating feeding band dynamics and the diffusion of metabolic waste from the feeding band into the food below. The model is parameterized using empirical data on multiple Drosophila laboratory populations adapted for larval crowding. The model simulates the effect of the amount of food and number of larvae on the evolution of greater competitive ability. It is also used to observe interactions among larval traits such as larval feeding rate, efficiency to convert food into biomass, critical size, waste tolerance, time to reach critical size and body size.

A further simulation study is focused on the effect of heritability and initial standing variation (sources of stochasticity in the model) on the evolutionary trajectories of larval traits responsible for competitive ability. I further extend the model to investigate patterns of early-late eclosing larvae and the larval traits they exhibit across densities. Results from this simulation study give a better understanding of various factors involved in the adaptation of *D. melanogaster* populations subjected to various scenarios of larval crowding.

Chapter 1

Introduction

The theory of density-dependent natural selection was verbally introduced by MacArthur (1962), MacArthur and Wilson (1967) to explore the evolution of phenotypes dependent on the population density. It is considered to be a critical link between ecological and evolutionary dynamics (Mueller, 1997). Over many years, this theory has been modified mathematically and studied experimentally to understand density effects on the evolution in great detail (Anderson & Arnold, 1983; Asmussen, 1983; Clarke, 1972; Mueller, 1997; Roughgarden, 1971; Santos, Borash, Joshi, Bounlutay, & Mueller, 1997). Early experimental studies also showed that selection at extreme densities causes selection for higher competitive ability since there is competition for limited resources (Joshi, Prasad, & Shakarad, 2001). Such competitive ability of an organism is composite phenotype determined by various life-history traits. Through these early studies, it was clear that density is a significant factor in determining the life-history of organisms which are essential for competitive ability. Competition plays a vital role in determining not only evolutionary outcomes of species but also ecological outcomes which affect population dynamics and interactions with other species (Case, 2000; Dey, Bose, & Joshi, 2012). Thus, in order to grasp a better understanding of density-dependent selection, exploring the effect of competitive ability on ecological and evolutionary dynamics becomes essential (Prasad & Joshi, 2003).

Over the last four decades, various *Drosophila melanogaster* laboratory populations have been used to study the evolution of life-history traits due to density-dependent selection. One of the first experimental evolution studies used r and K-selected pop-

ulations of *Drosophila melanogaster* (Mueller & Ayala, 1981)) to investigate the r- and K-selection theory by MacArthur (1962), MacArthur and Wilson (1967). In these populations, r-selection lines were maintained at low-density, giving density-independent selection. In contrast, lines for K-selection were maintained at extreme densities such that selection was density-dependent. As predicted by early mathematical models, these studies showed that r-selected populations favoured traits responsible for higher$r$-selected population growth rate at low densities but lower growth rate at extreme density. Bakker (1962), Burnet, Sewell, and Bos (1977) suggested that larval feeding rate, which is measured as retraction rate of cephalopharyngeal sclerites of the larva, is a critical factor in larval competitive ability. Experimental studies on r- and K-selection showed that K-selected populations have higher competitive ability along with increased larval feeding rate (Joshi & Mueller, 1988). This led to the conclusion that larval feeding rate is a good measure of competitive ability in *Drosophila* larvae. These populations could not predict classical density-dependent outcomes such as higher efficiency of food into biomass conversion (Mueller, 1990). Another problem with these populations was that r-selected populations were maintained in discrete generation cycles while K-selected populations were maintained in overlapping generations.

The successive experimental evolution studies were aimed at tackling questions raised in experimental studies mentioned above, by having a stage-specific density-dependent selection in a new set of *D. melanogaster* populations (described in Joshi and Mueller, 1993). In this long-term evolution study, a set of larval crowding (CU) population, another set of adult crowding (UC) population and one set of uncrowded (UU) *D. melanogaster* population were used. CU population adapted to larval crowding through a similar set of traits seen in K-selected populations. CU population had higher competitive ability than UU population at high-density which leads to increased pre-adult survivorship and decreased pre-adult development time (D. J Borash & Ho, 2001; Joshi & Mueller, 1993; Santos et al., 1997). Such competitive ability in CU larvae was due to increased feeding rate and increased nitrogenous waste tolerance at the cost of poor efficiency to convert food into biomass (Daniel J. Borash, Gibbs, Joshi, & Mueller, 1998; Joshi & Mueller, 1996; Shiotsugu, Leroi, Yashiro, Rose,

& Mueller, 1997). These results established a canonical view of density-dependent selection in *Drosophila* which argued that the evolution of greater competitive ability occured through increased feeding rate and metabolic waste tolerance at the cost of efficiency of food utilization (Joshi et al., 2001).

After the canonical view on adaptation to larval crowding was accepted widely, recent studies in different *Drosophila* species questioned this view. A subsequent study on adaptation to larval crowding involved *D. ananassae* and *D. nasuta nasuta* species of *Drosophila* which were wild-caught and subjected to long-term selection experiments similar to UU-CU populations (Nagarajan et al., 2016). Unlike CU population these were maintained at similar larval density but with decreased absolute number of eggs and total larval food. Due to adaptation to larval crowding in these populations, there was an increase in pre-adult survivorship at high-density and faster development compared to control at both low and high-density. In contrast to results from previous K and CU populations, these populations showed a reduction in time to reach critical size with no increase in larval feeding rate nor in nitrogenous waste tolerance (Nagarajan et al., 2016). Such reduced minimum critical feeding time was speculated to be due to increased efficiency of food into biomass conversion, which fit the *K*-selection theory of MacArthur and Wilson (1967). These surprising results were thought to be an outcome of several factors such as differences in species-specific genetic architect of traits responsible for larval competitive ability, differences in wild-caught populations and long-term laboratory populations, as well as differences in maintains of larval crowding suggesting the effect of ecological factors.

A long-term follow-up study on adaptation to larval crowding was performed using *Drosophila melanogaster* populations derived from UU populations to answer the questions raised from larval crowding studies of Nagarajan et al. (2016). In this study, a set of control populations (MB: Melanogaster Baseline) which had low larval density, and another set of populations (MCU: Melanogaster Crowded as larvae Uncrowded as adults) where larval stage was maintained at high-density similar to larval crowded populations of *D. ananassae* and *D. nasuta nasuta* (Sarangi, Nagarajan, Dey, Bose, & Joshi, 2016). MCU population showed the evolution of greater larval competi-

tive ability through a similar set of traits observed in the study of Nagarajan et al. (2016), i.e. decrease in the time to reach critical size without an increase in feeding rate. MCU larvae also did not differ in terms of metabolic waste tolerance but still showed faster pre-adult development time at both densities (Sarangi et al., 2016). In addition to these results, both MB and MCU populations showed a significant lower survivorship in the larval density of 1200 eggs / 6 ml food (CU-type culture) than in larval density of 600 eggs / 1.5 ml food (MCU-type culture) (Sarangi, 2013). MCU and CU population were derived from the same ancestry but still showed differences, indicating that ecological factors such as the overall number of eggs and total larval food, would be playing a significant role in determining which traits are selected for achieving greater competitive ability under larval crowding.

A subsequent study exploring ecological factors affecting adaptation to larval crowding involved two new set *D. melanogaster* populations derived from MB population. One set of these populations was CCU (Control Crowded as larvae Uncrowded as adults) population to address the effect of the absolute number of eggs and total food on evolution od larval competitive ability. Another set of populations was LCU (Larry Mueller Crowded as larvae Uncrowded as adults) population aimed at controlling for the food differences between CU and MCU populations since larval food used in these populations were banana and cornmeal medium respectively. In all these four *D. melanogaster* populations (MB, MCU, CCU and LCU) the adult stage was maintained in pretty much similar manner, whereas the details of larval stage maintenance are given in table 1.1 (Sarangi, 2018).

No.	Population	No. of eggs	Food volume	Vial dimensions
1.	MB	70	6 ml	9.5 cm h × 2.4 cm d
2.	MCU	600	1.5 ml	9.5 cm h × 2.4 cm d
3.	CCU	1200	3 ml	9.5 cm h × 2.4 cm d
4.	LCU	1200	6 ml	9.5 cm h × 2.2 cm d

Table 1.1: Larval stage maintenance in MB, MCU, CCU and LCU populations

After several generations of selection, CCU and LCU populations showed an increase in competitive ability and had higher pre-adult survivorship compared to MB popu-

lation at high densities (Sarangi, 2018). These two populations showed much higher feeding rate along with no difference in nitrogenous waste tolerance for achieving greater competitive ability, unlike MCU population. These results were interesting since MCU and CCU populations were maintained at the same larval density with varying total number of eggs and food. Sarangi (2018) also showed that feeding rate measured at the third instar stage of these larvae was dependent on the number of larvae present during larval feeding when assayed in slial (slide vials) treatment. When assayed inside culture vials, it was observed that the overall feeding rate of MCU population was the highest in all three high-density treatments in contrast to previous results performed on petri-dish. This result suggested that the ecological dynamics of the culture vial does play an essential role in determining competitive ability.

Inside a culture vial, larval feeding occurs only at the topmost part of total food present due to their inability to dig more (Godoy-Herrera, 1977). The available upper part of the total food is approx 1 cm in the height of a standard vial used in MB, MCU and CCU populations, and is called as 'feeding band' (Sarangi, 2018). Thus, the sufficient larval density, i.e. number of larvae per feeding band is double in CCU population than in MCU population. Another significant finding regarding ecological dynamics inside a culture vial was the diffusion of metabolic waste excreted by larvae from the feeding band into the food below (Sarangi, 2018). In MCU culture vials, the total amount of food is almost similar to the size of the feeding band. This lead to the speculation that in MCU culture vial, there is little-to-no diffusion of metabolic waste from the feeding band and food quality may decrease very rapidly during larval feeding affecting competitive ability. In CCU and LCU culture vials, diffusion of such metabolic waste occurs from the feeding band which leads could lead to a slower decrease in food quality affecting competitive ability in a manner different than in MCU culture vial.

In MCU, CCU and LCU populations, apart from pre-adult survivorship and feeding rate, other life-history traits such as dry weight at eclosion, development time also evolved differently (Sarangi, 2018). Experimental studies are limiting in order to understand above-mentioned ecological factors inside culture vials of these pop-

ulations. Thus, a computational simulation approach can be helpful to delve into ecological and evolutionary dynamics in these *Drosophila* populations.

In this thesis, I have presented a precursory stage-structured individual-based model to investigate adaptation to larval crowding in different crowding conditions based on the study of Sarangi (2018). This model is aimed at linking various ecological factors inside a culture vial, with the evolution of fitness-related traits and greater competitive ability through a combination of various larval traits. The later part of the model is also used to explore the role of initial standing variation in the population as well as heritability of larval traits, in determining the evolutionary trajectories to achieve greater competitive ability.

Chapter 2

Modelling Larval Stage in a Vial

Competition for food during the larval stage is determined by not only larval density but also ecological factors inside a food vial such as nitrogenous waste build-up, diffusion of waste in the food below, total food amount (Sarangi, 2018). Thus, in order to investigate the adaptation to larval crowding, it is crucial to understand the ecology of a vial in which the larval stage of Drosophila lab populations is maintained and replicating such environment during larval feeding becomes the first step in modelling the larval growth. Previous experimental studies on Drosophila in laboratory conditions have shown the pattern of the growth of larvae, excretion of nitrogenous waste, larval feeding behaviour in response to the various levels of larval crowding (Sarangi, 2018). Based on these experimental studies, I have created an individual-based model which considers larval trait parameters such as - feeding rate, efficiency to convert food into biomass, critical size and waste tolerance, to measure other traits like larval body size, development time, and survivorship.

2.1   Ecology of a Culture Vial

During larval feeding inside a vial, larvae can access only a certain amount of food from the total food available at a given time point. This is due to their inability to dig more to access food, and this accessible part of the food is referred as the feeding band (Godoy-Herrera, 1977; Sarangi, 2018). For simplicity, feeding band is taken as volume of food proportional to the width of the vial. In the model, I also assume this feeding band to be a constant volume of food in all types of culture vials till it reaches

the bottom of the vial (In LCU culture vials, fedding band is smaller). In the model, the growth of larvae is affected by waste build-up and food quality in the feedin band. I also consider a diffusion band which is a part of the total food below feeding band where some amount of waste can diffuse from feeding band at each time step. Fig 2.1 is the visualization of feeding band and diffusion band during larval feeding.
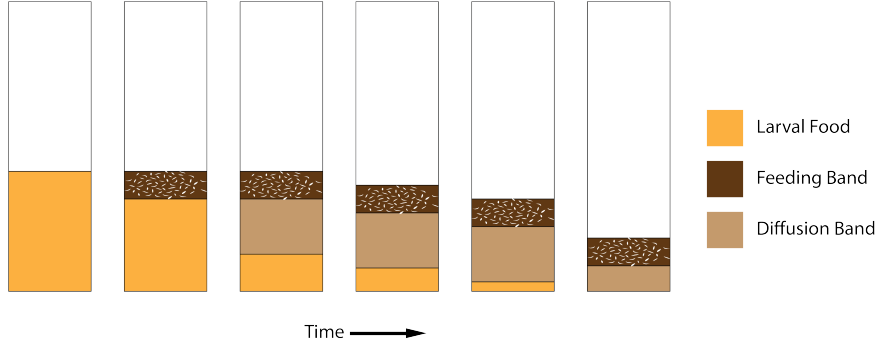
Figure 2.1: Ecological dynamics in a vial during larval feeding

2.2 Larval Stage Model

Each individual egg is assigned larval trait parameters from respective distributions with certain mean and variation given in table A.2. For a given amount of food and number of eggs, the model follows certain set of rules as described in fig 2.2 which are simulated in discrete time steps. The sex ratio within eggs is kept 1:1. Critical size and efficiency are taken as sexually dimorphic traits and are assigned depending on the sex of the individual larva. Critical size and efficiency of females are assumed to be 20% higher than that of males, so that females attain higher body size in the same time period as males but survivorship between sexes is same. (Joshi, Knight, & Mueller, 1996; Testa, Ghosh, & Shingleton, 2013)

The inital size of all larvae is same and the growth is determined by larval trait parameters such as initial feeding rate, efficiency, waste tolerance and critical size. The larval growth is divided into two stages determined by whether critical size is reached or not, These stages are called pre-critical and post-critical stage.

In pre-critical stage of the larva, feeding rate is a linear function of time, given as:

$$Fr_i(t) = fr_i + x_1 \cdot t$$

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Here,

fr_i: initial feeding rate of ith larva;

x_1: scaling parameter,

t: given time step;

$Fr_i(t)$: feeding rate at time t
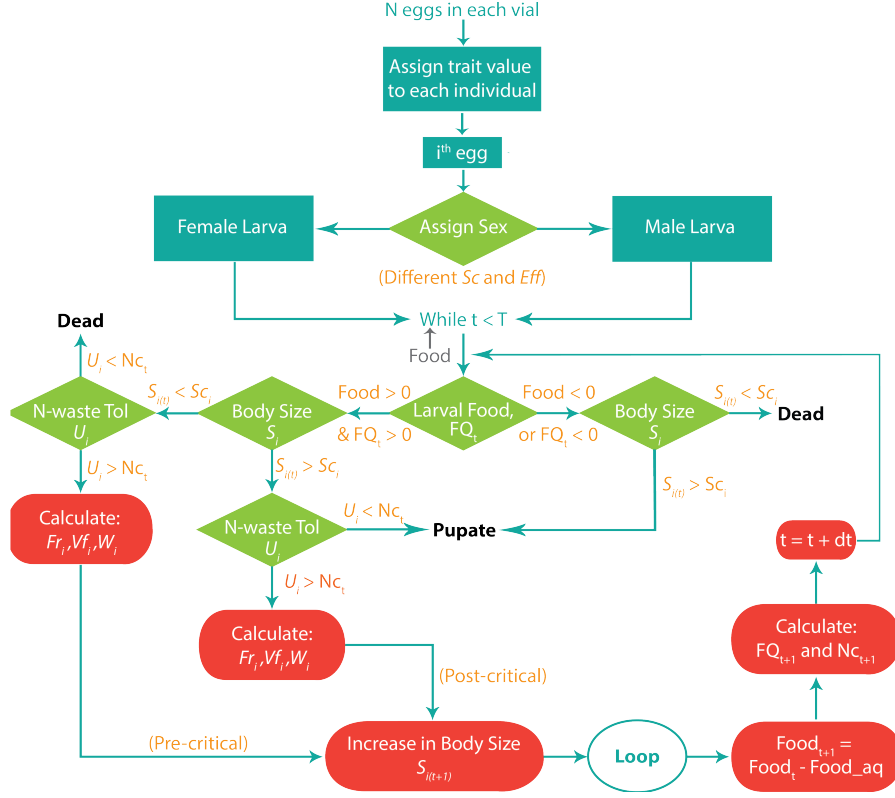
Figure 2.2: Flowchart of the larval stage in the model

Feeding rate stays constant During post-critical stage (see Santos et al., 1997). During pre-critical growth Volume of food taken in one bite is taken as constant $V_f(pre)$ and during post-critical growth it is $V_f(post) = 1.5 \cdot V_f(pre)$. Food consumed by all larvae at time step t is given as:

$$FoodEaten(t) = \sum_i food\_eaten_i(t) = \sum_i Fr_i(t) \cdot V_f$$

The increase in body size at time t is $S_i(t+1)$ and give as:

$$S_i(t+1) = S_i(t) + food\_eaten_i(t) \cdot \epsilon_i \cdot FQ_{fb}(t)$$

Here,

ϵ_i: efficiency to convert food eaten into biomass of ith larvae,

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$FQ_{fb}(t)$: food quality of the feeding band at time t

After feeding and utilizing food consumed at given time step, larva produces nitrogenous waste $waste\_prod_i(t)$. This affects the total waste produced by all the larvae after feeding:

$$WasteProd(t) = \sum_i waste\_prod_i = \sum_i [food\_eaten_i(t) \cdot (1 - \epsilon_i \cdot FQ(t))]$$

Based on this waste produced, total waste accumulated till time step t in feeding band and diffusion band is calculated considering k_d proportion of waste in the feeding band diffuses into diffusion band at each time step.

$$Waste_{fb}(t+1) = Waste_{fb}(t) + (1 - k_d) \cdot WasteProd(t) + \frac{FoodEaten(t) \cdot Waste_{db}}{dband}$$

$$Waste_{db}(t+1) = Waste_{db}(t) + k_d \cdot WasteProd(t) - \frac{FoodEaten(t) \cdot Waste_{db}}{dband}$$

Food quality of the feeding band at time step t is:

$$FQ_{fb}(t) = 1 - \frac{Waste_{fb}(t)}{fband}$$

If $FQ_{fb}(t) \leq 0$, it means that there is no food available to eat and feeding band contains only nitrogenous waste and larvae stop eating.

k_d is dependent on the food available in the vial and determines whether waste is diffused into the diffusion band. Its values are assigned at each time step as follows:

i k_d is a constant > 0 ... if $food > (fband + dband)$

ii $k_d = 0$ ... if $food \leq (fband + dband)$

Each larva feeds and increase the body size in each time step based on the conditions for food available ($food$), food quality ($FQ(t)$), critical size (sc_i) and waste tolerance (u_i) described in fig 2.2.

Values for all parameters used in the larval stage of the model, are given in table A.2, table A.5 and table A.1. These values were obtianed by calibrating survivorship, body size and development time results similar to the empirical results in various larval densities (Sarangi, 2018).

2.3   Simulations for Feeding Band Dynamics

Simulations are performed for trait values in table A.2 to observe the waste build up dynamics and food quality decrease in a food vial with different larval densities during larval feeding.

2.3.1   Waste build-up dynamics results

In fig 2.3, waste build in the feeding band throughout larval feeding at different larval densities is plotted. At low density i.e. 60 eggs / 6 ml food (MB culture), there is very little nitrogenous waste building up due to diffusion and plenty of food available below the feeding band at all time steps.
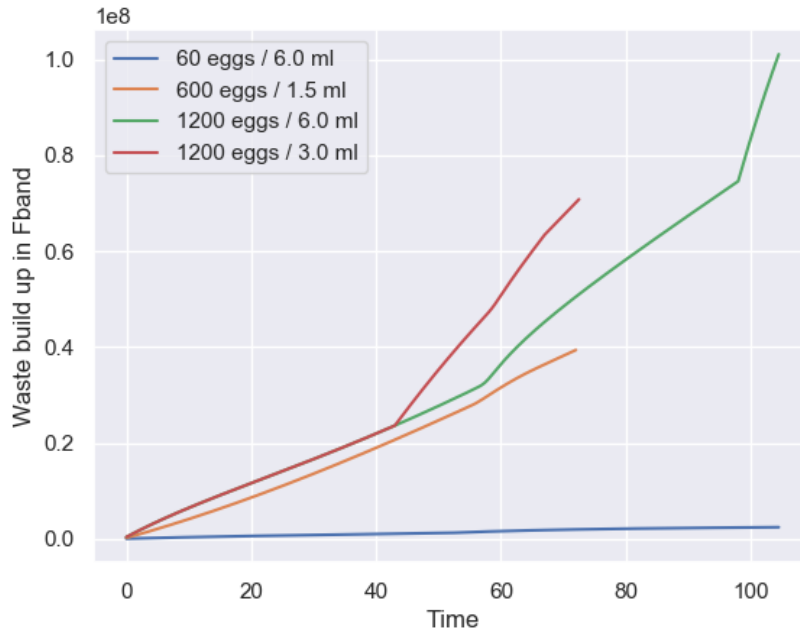
Figure 2.3: Waste build-up in the feeding band

High densities of 600 eggs / 1.5 ml food (MCU culture) and 1200 eggs / 3 ml food (CCU culture) show different patterns of waste build up in the feeding band, even though total larval density is equal. In MCU culture vial, there is very little food available below the feeding band, thus diffusion does not occur and waste build in the feeding band increases gradually. In CCU culture vial, waste build-up is almost in same qauntity as in MCU culture in earlier stage, even though effective larval density is double (number of larvae per feeding band). This is due to the availablilty of

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food below feeding band in CCU culture where waste can diffuse. After approx. 40th time step, diffusion stops and waste from diffusion band enters feeding band in more quantity, thus giving a sudden increase in the waste build rate.

LCU culture vial (1200 eggs in 6 ml food) also shows pattern of waste build in the feeding band similar to CCU culture vial, but shows increase in the rate of waste build up approx. after 60th time step. This is due to the food is still available below the feeding band. At approx. 100th time step in LCU culture vial shows even more increase in the rate of waste build because diffusion band touches the bottom and starts shrinking.

2.3.2   Food quality dynamics results
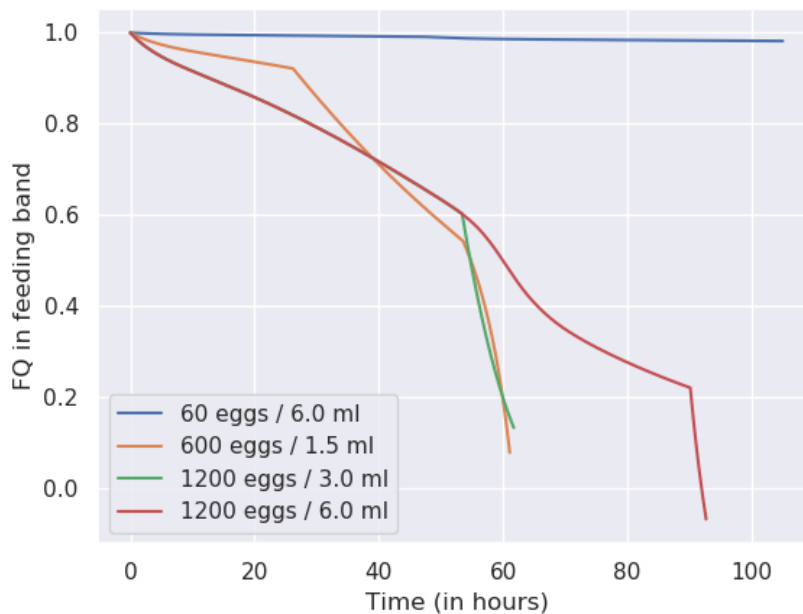
Figure 2.4: Change in the food quality of feeding band

Fig 2.4 shows the decrease in the quality of the food present in the feeding band. Food quality being negatively correlated with the amount of waste build-up in the feeding band, it shows patterns similar to waste build-up during larval growth in all crowding condtions. Since food quality affects body size increment at each time step. Body

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size increment between 40th and 60th time step is completely different in MCU and CCU cultures even though there larval density is equal. In LCU culture, decrease in food quality is simlar to CCU culture till 60th time step but later decreases gradually unlike CCU. This gradual decrease is due to gradual waste diffusion into the available food below the feeding and diffusion band. Once diffusion band hits the bottom at 90th time step, food quality decreases rapidly.

Chapter 3

Interplay between Larval Trait Parameters

In the larval stage model, trait parameters used are initial feeding rate, efficiency, critical size and waste tolerance. These parameters can not be measured directly via experimental approaches. However, their effects on other larval traits such as body size, feeding rate at the third instar, development time can be measured experimentally. Here, I explore how larval trait parameters interact with each other and affect body size, time to reach critical size, feeding rate at critical size and survivorship. Since the feeding rate in the model stays constant after reaching critical size, it can be taken as a proxy for feeding rate at the third instar stage. Also, time to reach critical size is taken as a proxy for development time since time period between critical size, and pupation is taken as a constant (see Santos et al., 1997). The larval stage is simulated to obtain body size, development time, final feeding rate (at critical size) and survivorship in MB, MCU and CCU cultures for each combination of initial feeding rate, efficiency and critical size from a respective range of mean trait values. Here, the effect of waste tolerance is ignored on the body size, development time, final feeding rate and survivorship since no significant effect was observed. Using experimental data and these simulation results, the best combination of trait values are obtained, which represents ancestral trait values for each population. Traits measured using these trait values represent MB flies from the experimental data, and these trait values are used in further simulations.

3.1   Initial Feeding Rate and Efficiency

All simulation results show that the larval body size, development time, final feeding rate and survivorship are dependent on the larval density. In MCU and CCU culture, overall body size and survivorship are lesser while development time and final feeding rate are always higher for the same range of trait values than in MB culture (see fig 3.1 - fig 3.12). The larval body size is positively correlated with both initial feeding rate and efficiency at low density (MB culture). In contrast, at high densities (MCU and CCU cultures) it is positively correlated only with efficiency (see fig 3.1). In MCU culture, body size is not affected by initial feeding rate, whereas initial feeding rate gives lesser body size in CCU culture. Fig 3.2 shows a negative correlation of development time, i.e. time to reach critical size with both initial feeding rate and efficiency at all larval densities. Survivorship is logistically dependent on efficiency only in MCU and CCU cultures (see fig 3.3). In MCU culture, survivorship does not show any dependence on initial feeding rate, but it shows a slight negative correlation with initial feeding rate in CCU culture. At all larval densities, final feeding rate is positively correlated with initial feeding rate and negatively with efficiency (see fig 3.4). In MCU and CCU culture, the final feeding rate shows positive dependence with efficiency, which increases further with higher initial feeding rate.

(a) MB culture   (b) MCU culture   (c) CCU culture
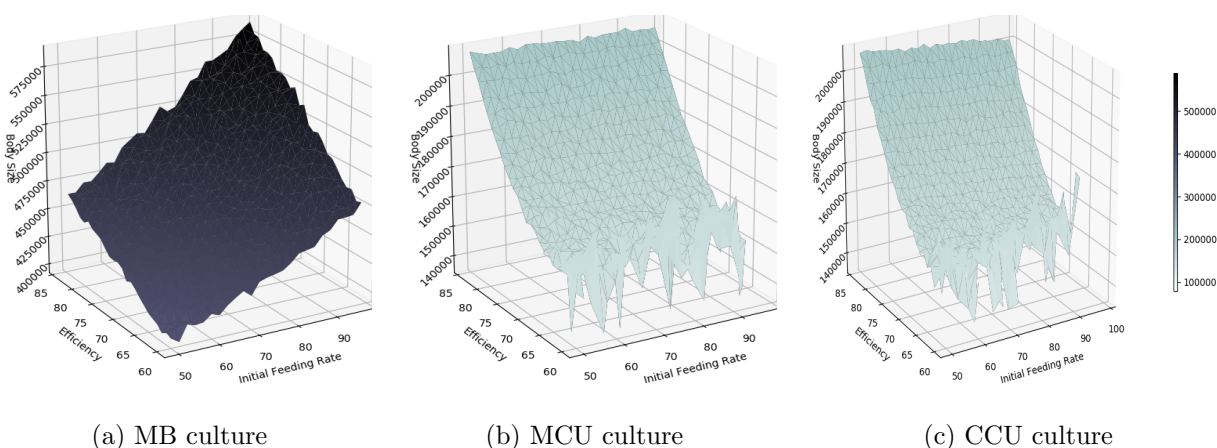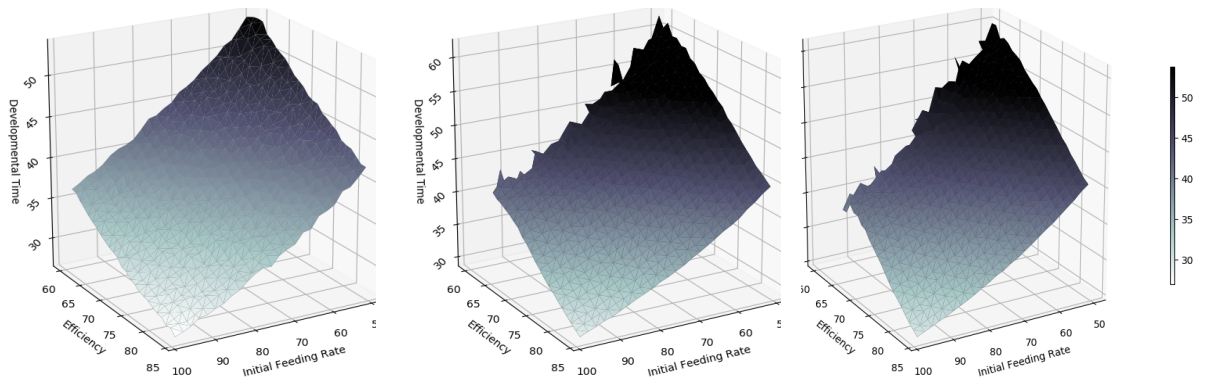
Figure 3.1: Effect of initial feeding rate and efficiency on body size

(a) MB culture   (b) MCU culture   (c) CCU culture

Figure 3.2: Effect of initial feeding rate and efficiency on development time
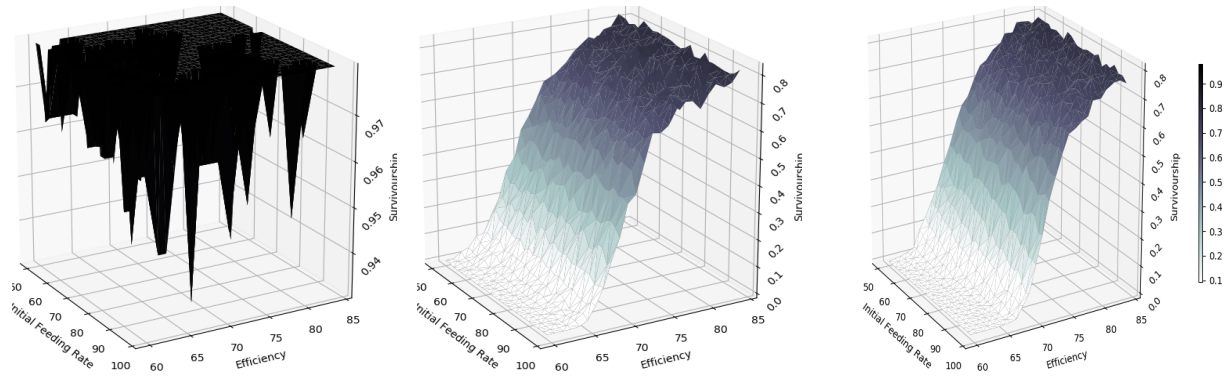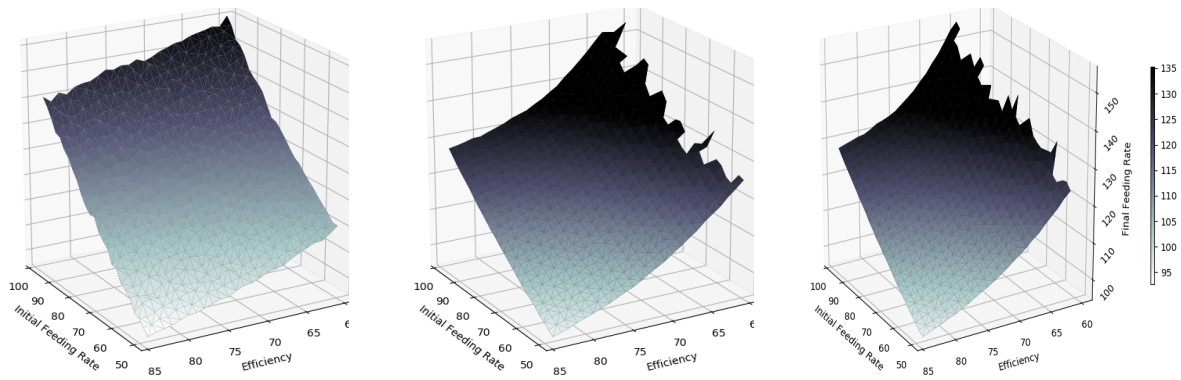

(a) MB culture   (b) MCU culture   (c) CCU culture

Figure 3.3: Effect of initial feeding rate and efficiency on survivorship

(a) MB culture   (b) MCU culture   (c) CCU culture

Figure 3.4: Effect of initial feeding rate and efficiency on final feeding rate

3.2 Initial Feeding Rate and Critical Size

In simulations with varying mean trait values of initial feeding rate and critical size, all traits show a similar pattern with a varying density as seen with previous simulations. The larval body size shows similar correlations with initial feeding rate and critical size in MB and MCU culture, as seen in simulations varying initial feeding rate and efficiency (see fig 3.5). In CCU culture, the body size is negatively correlated with initial feeding rate only for smaller values of critical size. However, it is not affected by initial feeding rate at larger critical size values. Fig 3.6 shows a negative correlation of development time with initial feeding rate, but a positive correlation with critical size in all culture vials. Survivorship is logistically dependent on critical size only in MCU and CCU culture. In MCU and CCU cultures, survivorship shows a slight negative correlation with initial feeding rate (see fig 3.7). At all larval densities, final feeding rate is positively correlated with both initial feeding rate and critical size (see fig 3.8).

(a) MB culture   (b) MCU culture   (c) CCU culture
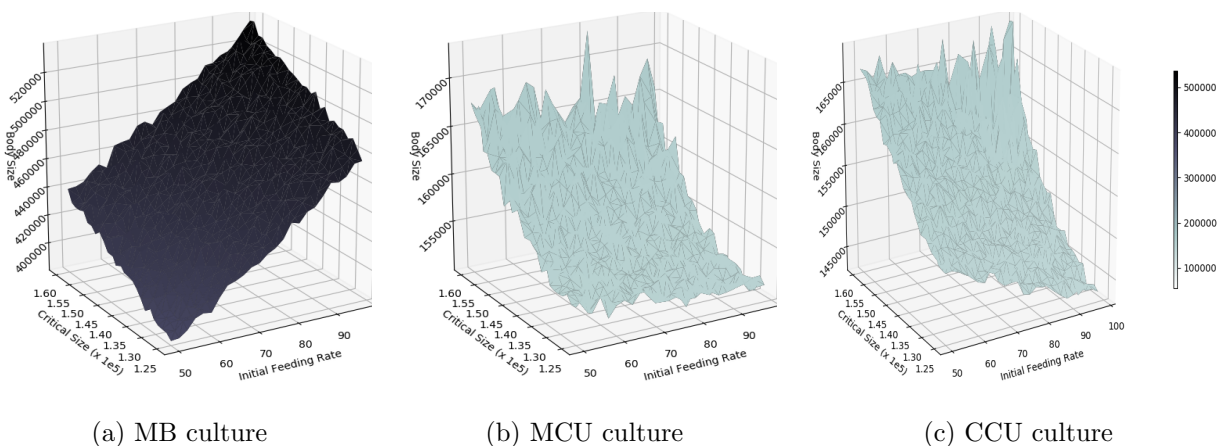
Figure 3.5: Effect of initial feeding rate and critical size on body size

(a) MB culture   (b) MCU culture   (c) CCU culture

Figure 3.6: Effect of initial feeding rate and critical size on development time

(a) MB culture   (b) MCU culture   (c) CCU culture
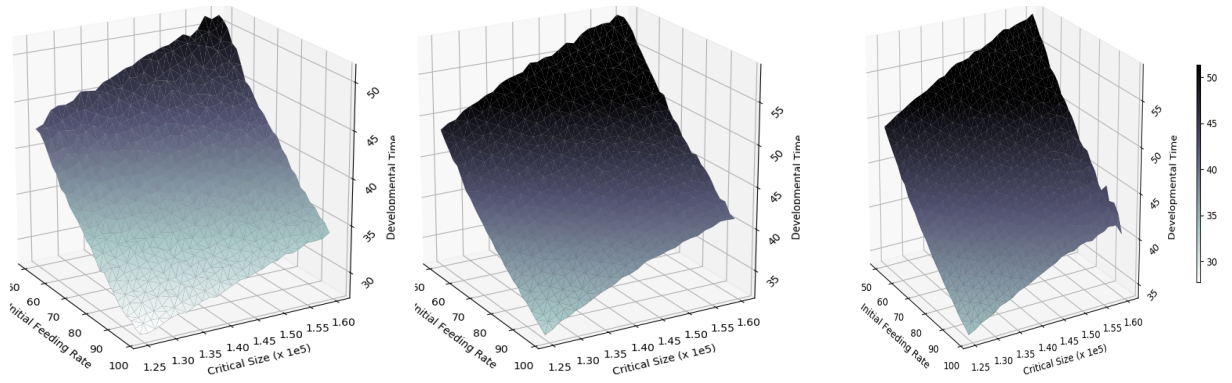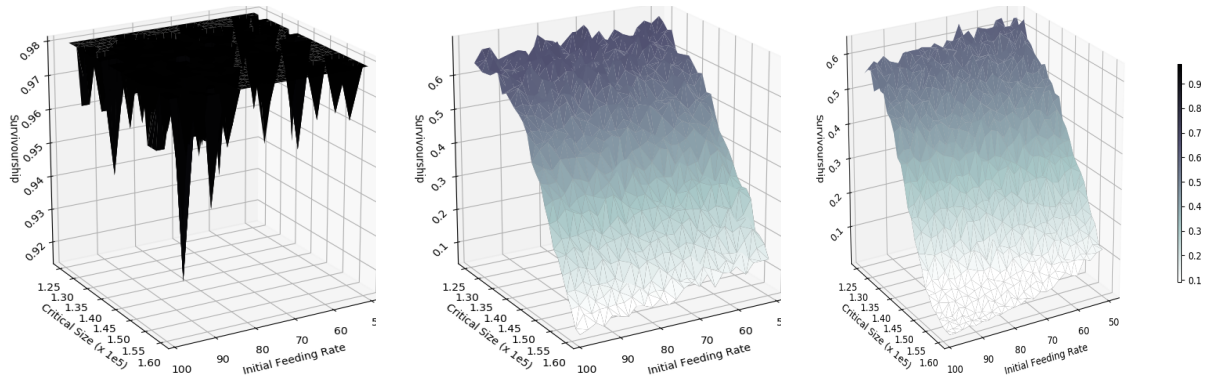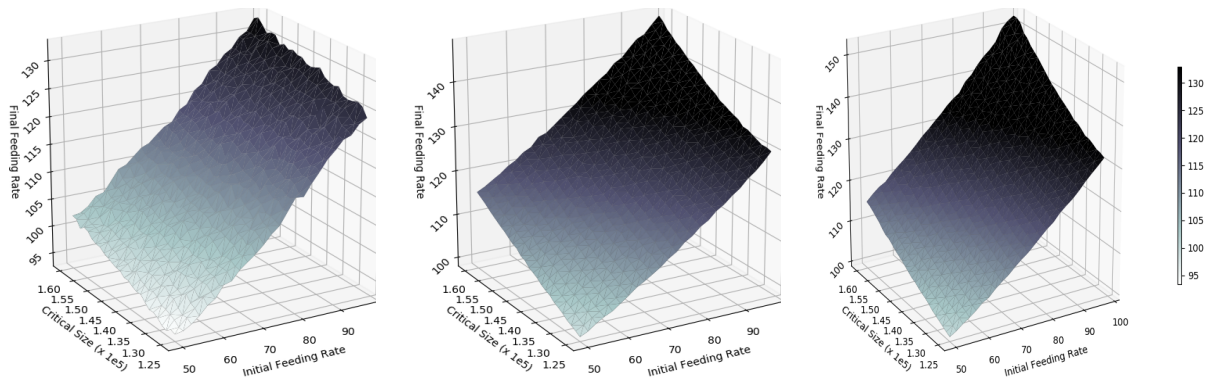
Figure 3.7: Effect of initial feeding rate and critical size on survivorship

(a) MB culture   (b) MCU culture   (c) CCU culture

Figure 3.8: Effect of initial feeding rate and critical size on final feeding rate

3.3 Critical Size and Efficiency

In simulations varying mean trait values of critical size and efficiency, all larval traits measured show again a similar pattern with density. The larval body size shows similar correlations with critical size and efficiency in MB culture, as seen in previous simulations, varying initial feeding rate and efficiency (see fig 3.9). In MCU and CCU cultures, the body size is positively correlated with critical size only for smaller values of efficiency. However, it is not affected by a critical size at larger efficiency values. Fig 3.10 shows a negative correlation of development time with efficiency, but positive correlation with critical size at all densities. Survivorship is again logistically dependent on efficiency only in MCU and CCU culture. In MCU and CCU cultures, survivorship also shows a negative correlation with critical size at lower values of efficiency (see fig 3.11). At all larval densities, final feeding rate is positively correlated with critical size and negatively with efficiency (see fig 3.12).

(a) MB culture   (b) MCU culture   (c) CCU culture
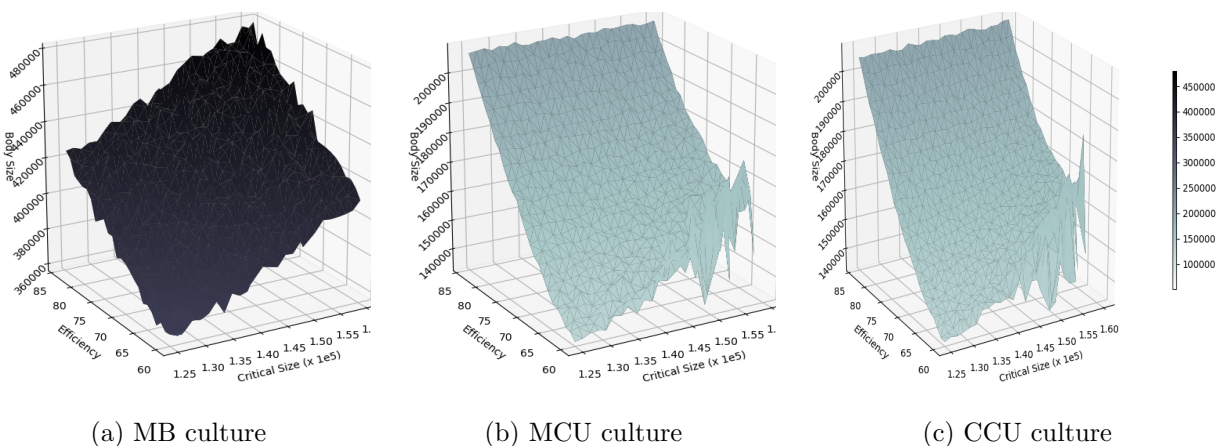
Figure 3.9: Effect of critical size and efficiency on body size

(a) MB culture   (b) MCU culture   (c) CCU culture
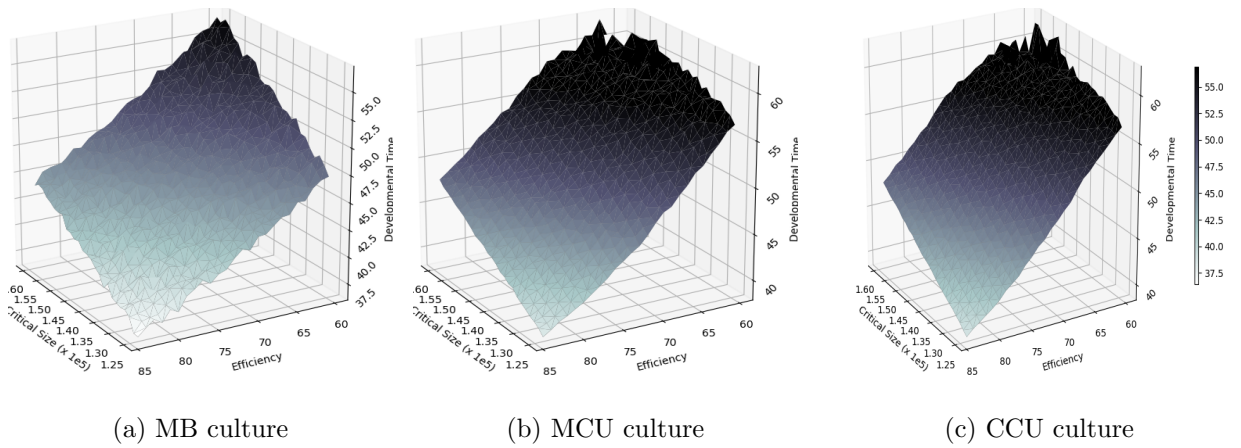
Figure 3.10: Effect of critical size and efficiency on development time

(a) MB culture   (b) MCU culture   (c) CCU culture
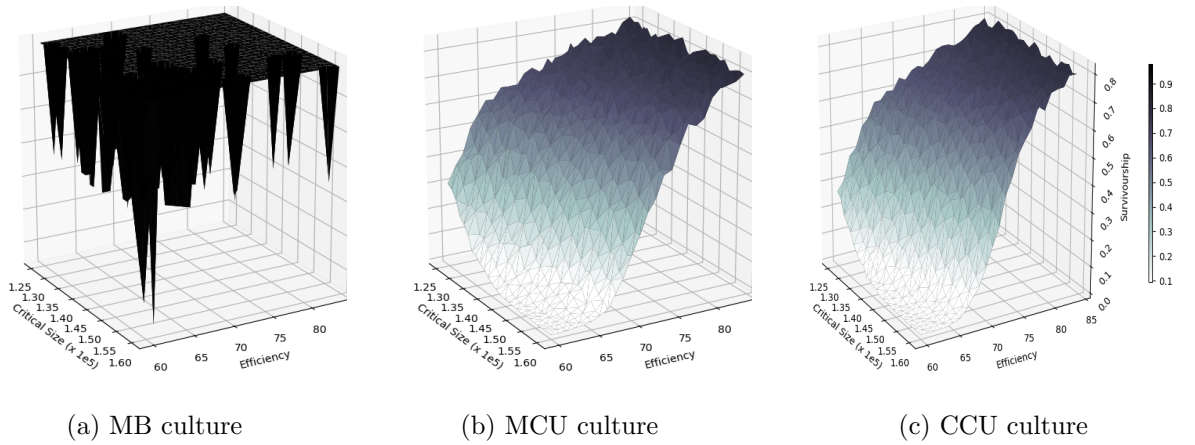
Figure 3.11: Effect of critical size and efficiency on survivorship

(a) MB culture   (b) MCU culture   (c) CCU culture

Figure 3.12: Effect of critical size and efficiency on final feeding rate

Chapter 4

Modelling Evolution of Life-history Traits

4.1 Modelling Adult Stage

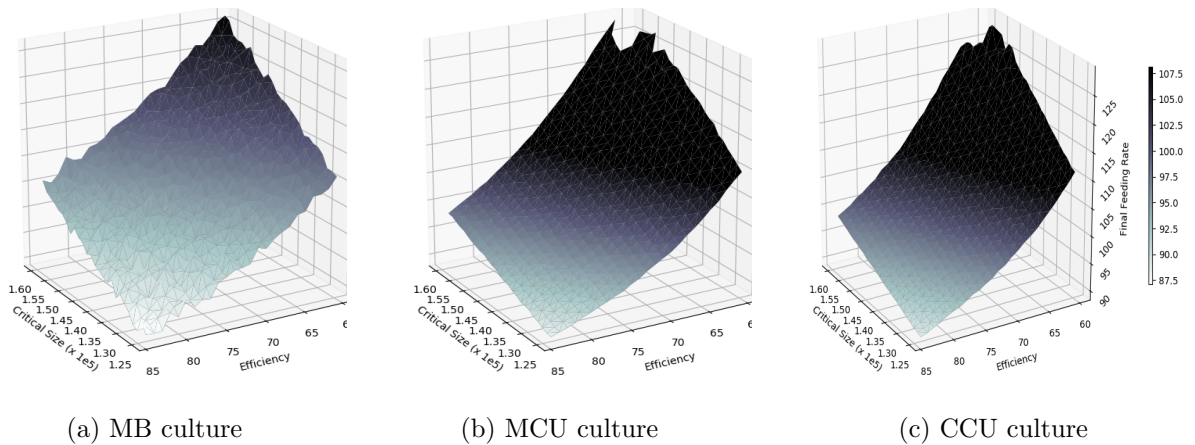After modelling the larval stage and calibrating, I developed the model for the pupal and adult stage of *Drosophila* life cycle. After the larval stage, surviving individuals go through the pupal stage, where some of them undergo pupal mortality. The adult stage includes randomly choosing surviving adults from all replicate vials of the pupal stage, matings, and inheritance of larval trait parameters from parents to offspring. Female is mated once with random male chosen (with replacement) from the adult population ($n = 2400$) for simplicity. From all the offspring produced, eggs are chosen at random for the next generation with numbers respective to the crowding environment maintained.

4.1.1 Pupal stage

After collecting all the surviving individuals from the larval stage, a probability of death during the pupal stage is assigned to each survived larva. This probability is dependent on the amount of waste accumulated in the body while consuming food during the larval stage. This probability is given as:

$$P_M(i) = 1 - exp(-(W_{acum}(i) \cdot x_3)^2)$$

Here,

P_M: probability of dying during pupal stage;

$W_{acum}(i)$: waste accumulated by i^{th} larva during larval stage;

x_3: scaling parameter.

4.1.2 Fecundity

After each mating, the number of eggs produced for a female is derived from the fecundity equation based on the model of (Tung, Rajamani, Joshi, & Dey, 2019). Fecundity is taken as a function of body size of the female and adult nutrition parameter (the amount of yeast provided). Fecundity of an ith female is given as:

$$Egg_i = Nut \cdot x_4 \cdot \log (x_5 \cdot s_i)$$

Here, s_i: body size of the ith female;

Egg_i: number of eggs laid by the female in a mating;

Nut: adult nutrition i.e. the amount of yeast provided;

x_4, x_5: scaling parameters.

4.1.3 Inheritance

Larval trait parameters (initial feeding rate, efficiency, waste tolerance and critical size) are inherited from parents to offspring produced by each female using mid-parent value. The mid-parent value, i.e. the average of mother and father for each larval parameter of all offspring, is calculated. This mid-parent value is taken as a mean of a normal distribution with a fixed standard deviation for respective trait parameters. The standard deviation in this normal distribution determines the heritability of the mid-parent value, and it is considered to be different for each trait parameter. Trait parameters of the offspring are assigned as:

$$T_i \in N(mpv_T, \delta_T)$$

Here,

T_i: trait parameter assigned to i^{th} offspring from a mating;

mpv_T: mid-parent value of of the trait T for a given mating;

δ_T: heritability of mid-parent value of the trait T;

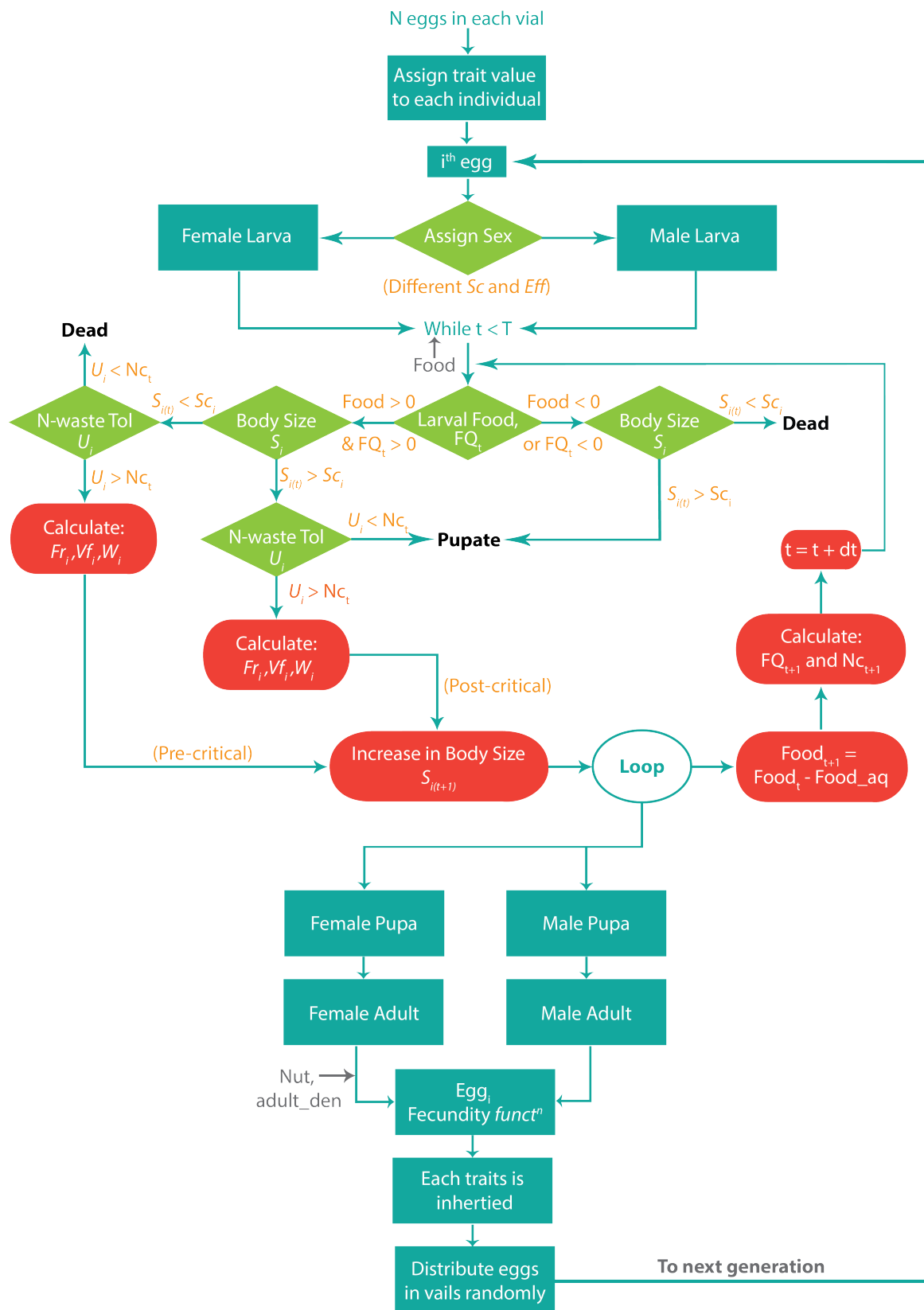$N(mpv, \delta)$: normal distribution with mpv as mean and δ as standard deviation.

Figure 4.1: Complete flowchart of the model

4.2 Evolution of Larval Trait Parameters

Using values for all parameters given in table A.5 and table A.1, the entire model is simulated for 100 generations with 10 replicates for MB, MCU and CCU cultures (see fig 4.1). This first set of simluations on the evolutionary part is aimed at investigating differences in the evolution of competitive ability in MCU and CCU populations which have same larval density but different ecological dynamics. In the model, all larval trait parameters are taken from independent distribution, and there is no correlation between them (see table A.2). Timeseries for these traits of surviving adult individuals are plotted with 95% CI.

In MB culture, being control population, none of the trait parameters evolve over time (see fig 4.2 - 4.5). Initial feeding rate in high-density cultures increase over generations at a similar rate, but initial feeding rate is higher always in CCU culture than in MCU culture. Efficiency shows a similar trend in high-density cultures, i.e. increase over generations at a similar rate which is always higher in CCU culture always than in MCU culture. Critical size in CCU culture is always lower than in MCU culture. Waste tolerance does not evolve in all of the culture populations since there is no significant effect of change in waste tolerance value on competitive ability.
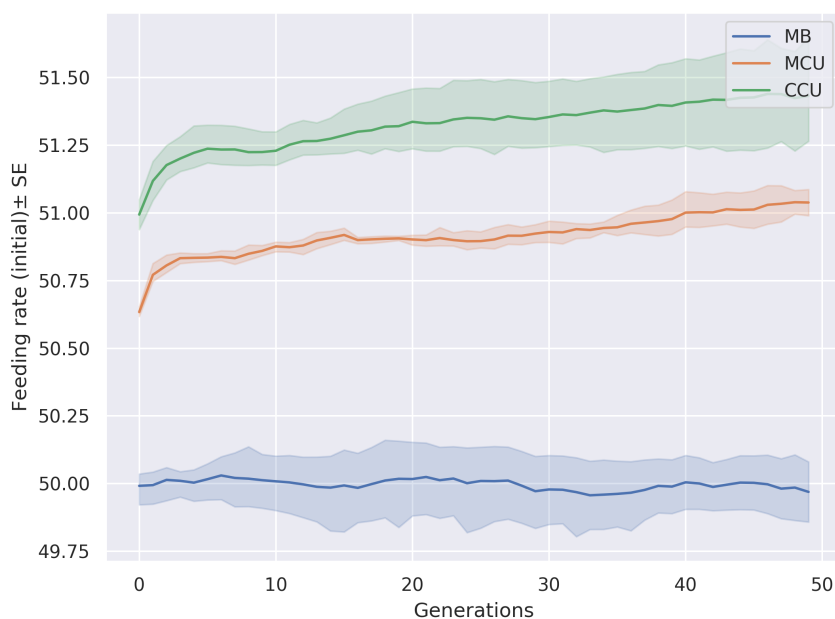

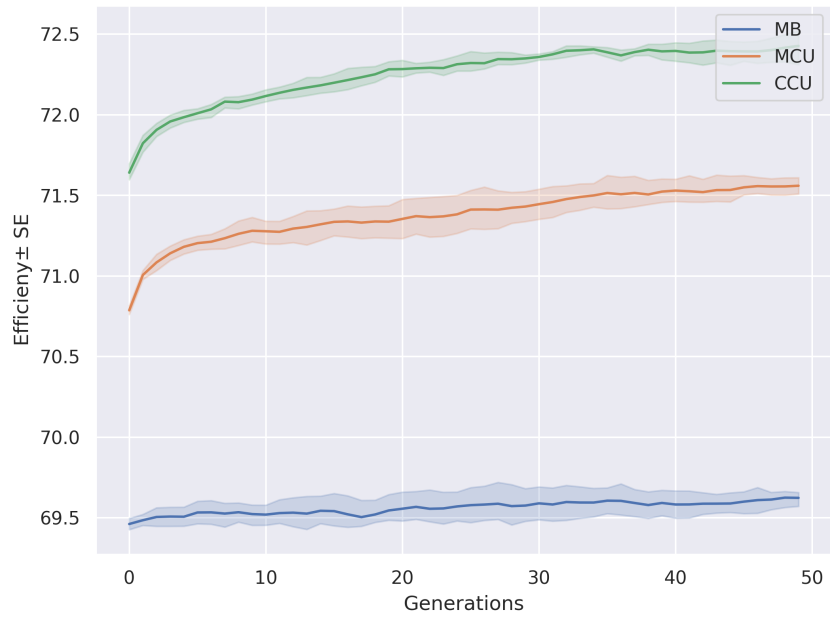Figure 4.2: Timeseries for initial feeding rate
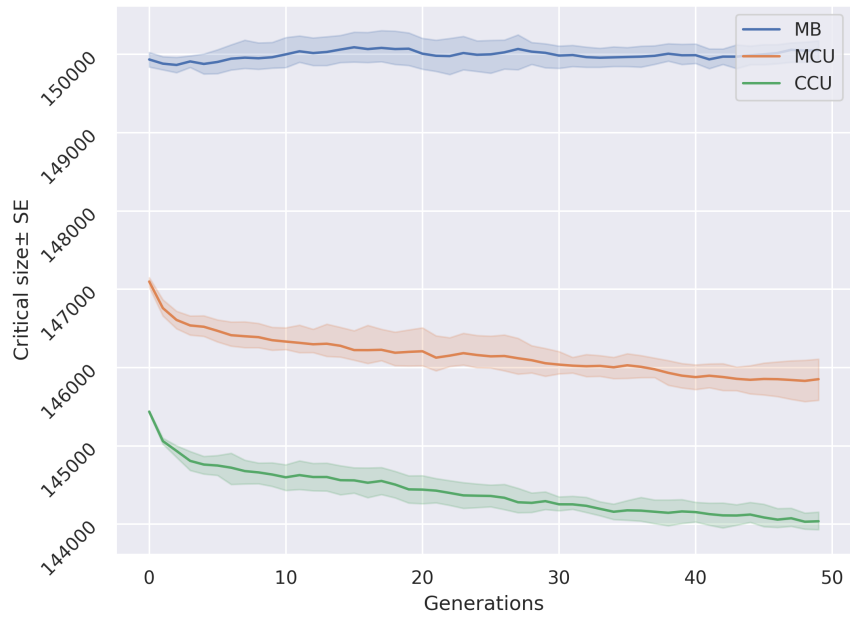
Figure 4.3: Timeseries for efficiency

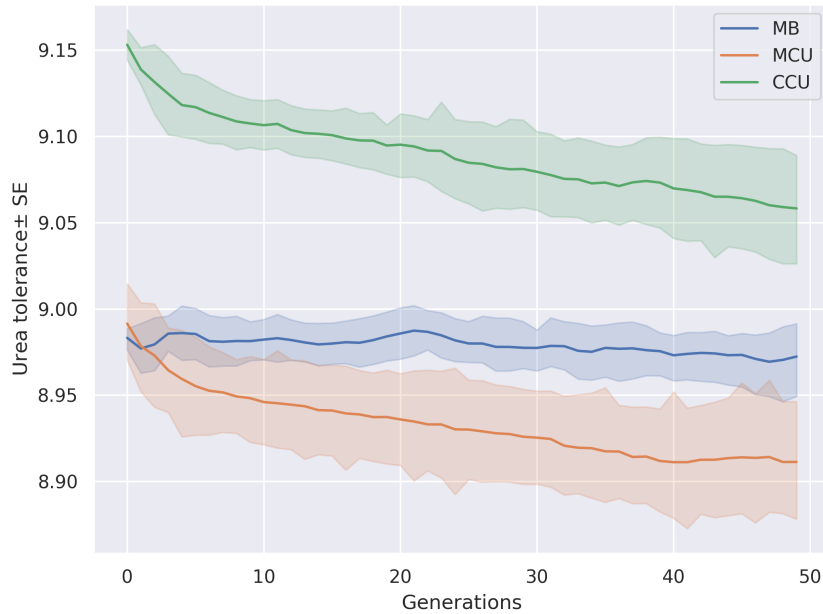Figure 4.4: Timeseries for critical size

Figure 4.5: Timeseries for waste tolerance

4.3 Evolution of Fitness-related Life-history Traits

From previous simulations on the evolution of larval trait parameters related to competitive ability, mean trait values of above-mentioned larval trait parameters are obtained for MB, MCU and CCU populations after 50 generations. Using mean trait values of initial feeding rate, efficiency, critical size and waste tolerance of respective populations; the larval stage model is simulated in order to investigate the evolution of fitness-related larval traits (replicates = 10). These traits include larval body size, survivorship, and time to reach critical size measured across MB, MCU and CCU culture densities (see fig 4.6 - 4.8).

At higher densities, all fitness-related traits show the effect of larval density. Body size fand survivorship or all three populations is lesser at MCU culture (600 eggs / 1.5 ml) and CCU culture (1200 eggs / 3 ml) (see fig 4.6). MCU larvae show a larger body size than CCU larvae at low density. Survivorship is higher in MCU culture than in CCU culture for all three populations (see fig 4.7). Survivorship has increased in MCU and CCU population at high densities, such that CCU larvae survive more than MCU larvae in both MCU and CCU cultures. Time to reach critical size is higher at higher densities for all three populations (see fig 4.8).

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MCU larvae are able to reach critical size faster than CCU at all densities. Both MCU and CCU larvae reach evolved faster critical feeding time. The majority of these simulation results are similar to the empirical data, except for the survivorship results of CCU larvae (Sarangi, 2018).
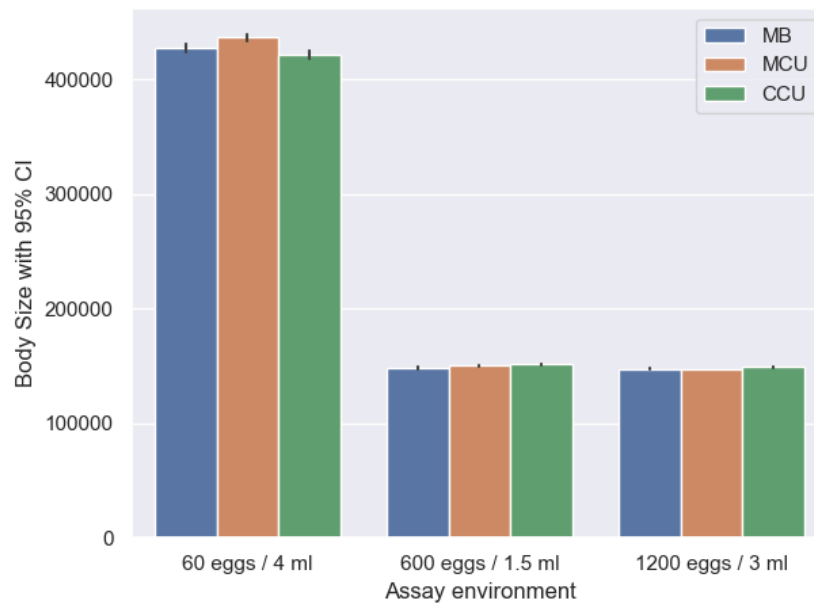

Figure 4.6: Mean body size of MB, MCU and CCU populations at 50th generation in three different larval densities
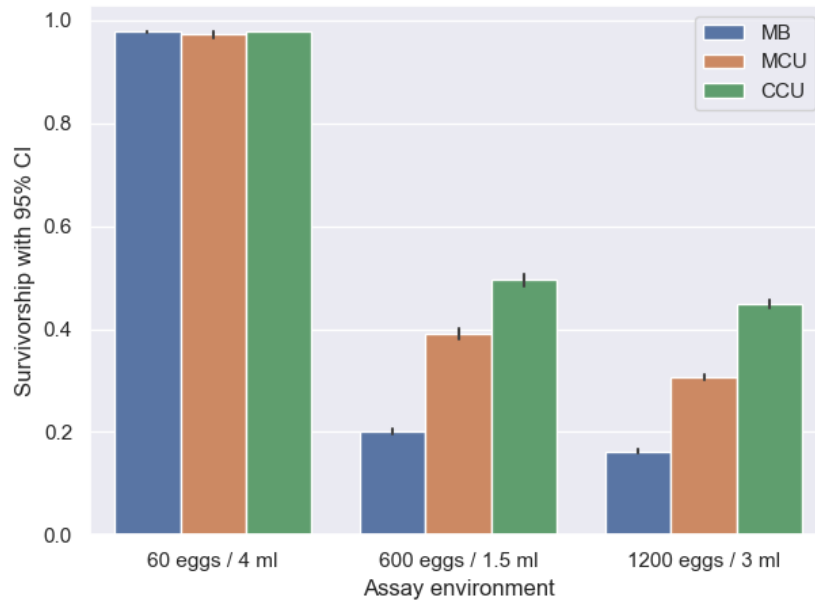
Figure 4.7: Mean survivorship of MB, MCU and CCU populations at 50th generation in three different larval densities
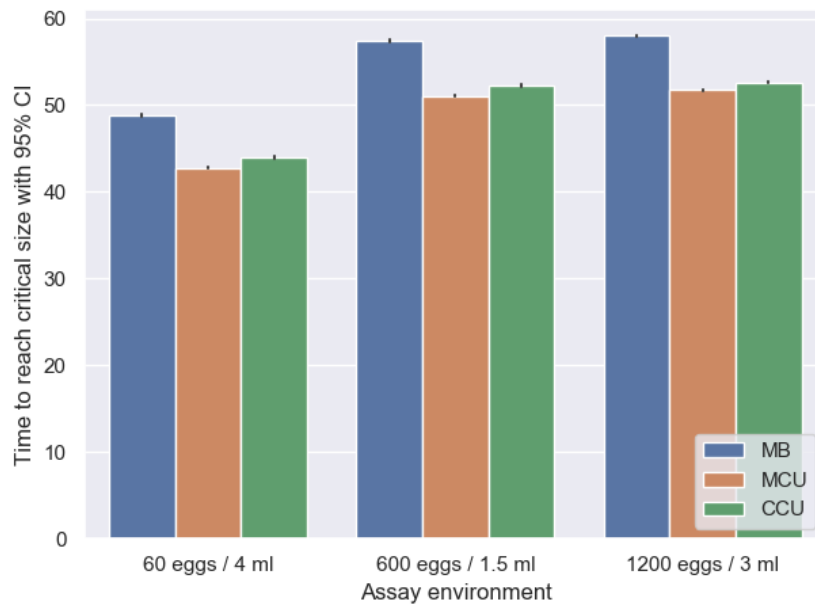

Figure 4.8: Mean time to reach critical size of MB, MCU and CCU populations at 50th generation in three different larval densities

Chapter 5

Effects of Variation on the Evolution of Larval Trait Parameters

Stochasticity in the model comes from initial standing variation in larval trait parameters as well as from heritability of each trait parameter during the inheritance of those traits. The results from simulations show how these sources of variations play an important role in determining the evolutionary routes taken to increase fitness and achieve greater competitive ability.

5.1   Variation in the Initial Distribution of Larval Trait Parameters

In the initial distribution of each trait value, the variation comes from the standard deviation given for each distribution of the traits like feeding rate, waste tolerance, critical size and efficiency. The initial standing variation in these trait distributions determines the maximum mean trait value that can be achieved to increase the fitness. Simulations were performed for given fixed mean trait values but varying their respective initial standing variation in MB, MCU and CCU cultures (generations = 50, replicates = 5). These simulations were aimed at investigating how these variations interact in order to obtain maximum fitness. In fig 5.1 - 5.3, differences of the

mean trait values of the population at 50th generation and 0th generation are plotted for different combinations of initial variation in trait values. The initial standard deviation for a trait is taken as a certain percentage of its respective mean trait value at 0th generation given in table A.2.

These results show that differences in variation of these trait parameters give different mean trait values at 50th generation across different crowding densities. Overall there is no significant effect of initial variation in trait parameters on the mean trait values in MB culture. Higher initial variation in initial feeding rate and efficiency give higher mean initial feeding rate and mean efficiency respectively after 50th generation in MCU and CCU cultures without showing any interaction (see fig 5.1). There is no difference between the mean trait value of efficiency of MCU and CCU cultures. Mean initial feeding rate evolved in MCU culture is lesser than in CCU culture only when initial variation in this trait is high(see fig 5.2). There is no significant effect on the mean critical size at 50th generation due to initial variation in initial feeding rate and critical size at all densities. In Fig 5.3, initial variation in efficiency and critical size both interact with each other, which gives a significant difference in mean efficiency between MCU and CCU cultures. At lower initial variation of efficiency, there is no effect of initial variation in critical size on the mean efficiency (see fig 5.3). This pattern is similar in MCU and CCU cultures. Higher initial variation in the efficiency and higher initial variation in critical size, both together lead to a difference in mean efficiency across MCU and CCU culture. At higher initial variation in efficiency but lower variation in critical size, there is no significant difference in mean efficiency across MCU and CCU culture. Fig 5.3 shows the interaction of initial variation in efficiency and critical size in achieving higher efficiency across MCU and CCU cultures. There is no effect of these variations on mean critical size. There was no effect of these variations on waste tolerance, and the graphs are given in appendix A.
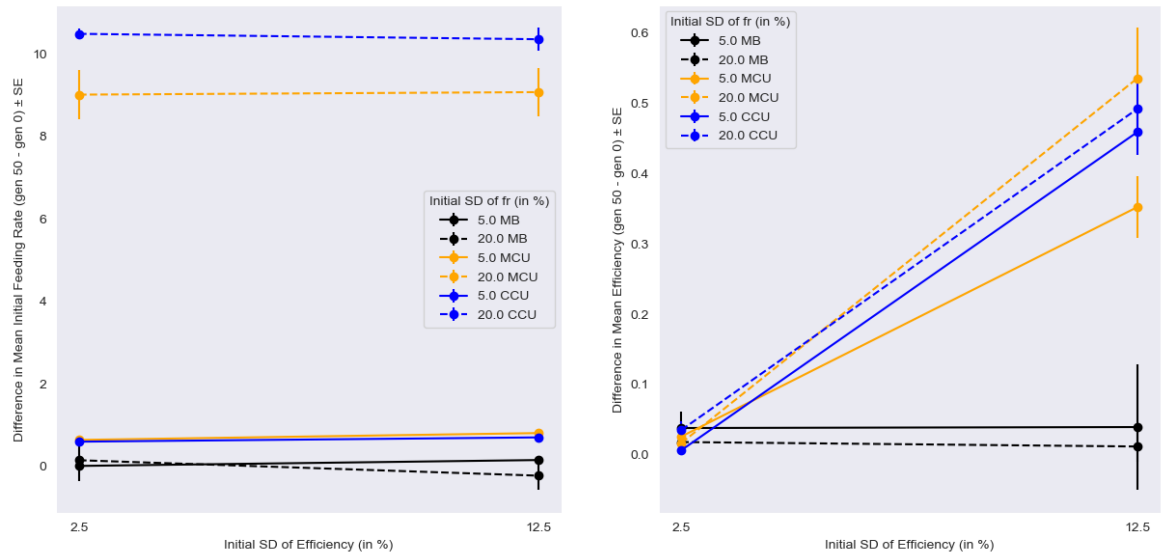
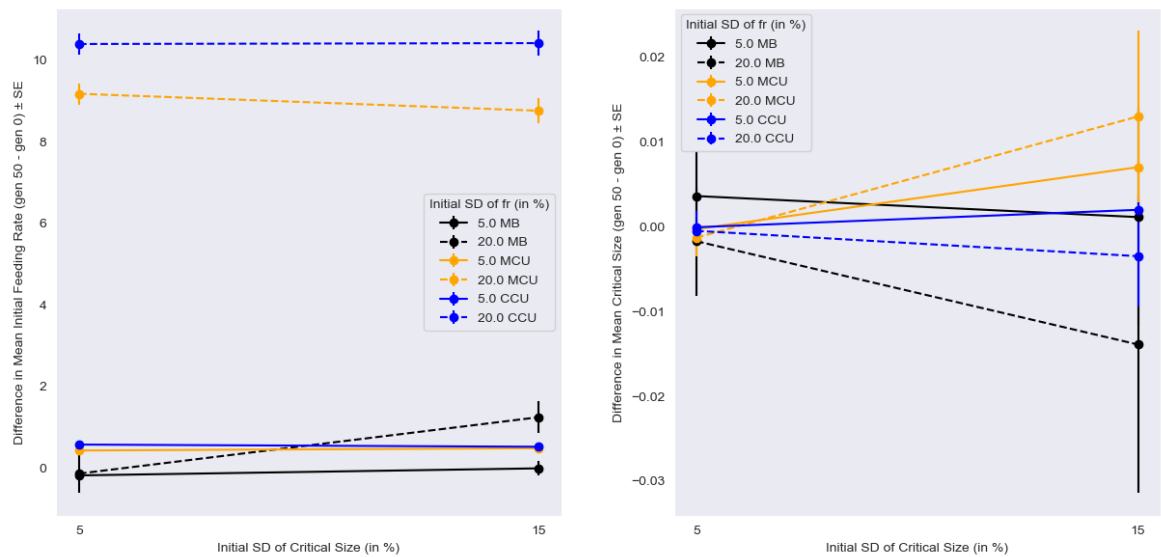Figure 5.1: Effect of initial variation in initial feeding rate and efficiency.

Figure 5.2: Effect of initial variation in initial feeding rate and critical size.
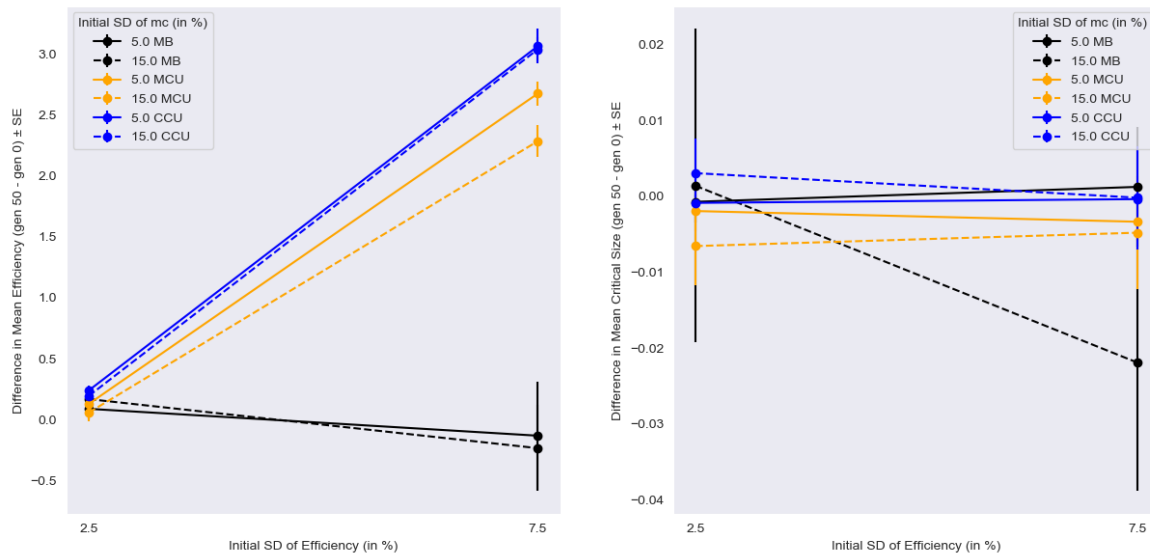
Figure 5.3: Effect of initial variation in critical size and efficiency.

5.2 Heritability of Larval Trait Parameters

During the inheritance of trait parameters from parents to offspring, the variation in the trait value of offspring from the parents comes from heritability of that trait parameter. In the model, trait values are assigned to offspring from a normal distribution around mid-parent value as mean, and a certain standard deviation (ω). This standard deviation, ω, in the respective trait distribution is taken as a measure of heritability for that trait. It is taken as a certain percentage of the mean value of the respective trait parameter at 0th generation. Higher the standard deviation, lesser is heritability for that trait parameter. For fixed initial conditions, simulations are performed with varying ω for combinations of trait parameters across MB, MCU and CCU cultures (see table A.2, generations = 50, replicates = 5). The results from these simulations are plotted similar to initial variation plots (see fig 5.4 - 5.6).

Results from these simulations show that initial feeding rate, efficiency and critical size do not show any significant effect of heritability of these trait parameters in MB culture. Higher heritability of initial feeding rate leads to higher mean initial feeding rate in both MCU and CCU culture. In MCU and CCU cultures, mean initial feeding rate decreases with a decrease in the heritability of efficiency only for higher heritability of initial feeding rate. Otherwise, such interaction of mean initial

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feeding rate with the heritability of efficiency is only observed in CCU culture at low heritability of initial feeding rate (fig 5.4). The only significant difference in mean efficiency is between MCU and CCU culture when heritability of efficiency is less. Mean critical size is not affected by heritability of these two trait parameters at all densities. Lower heritability of efficiency also seems to cause an increase in mean waste tolerance without any interaction in all cultures.
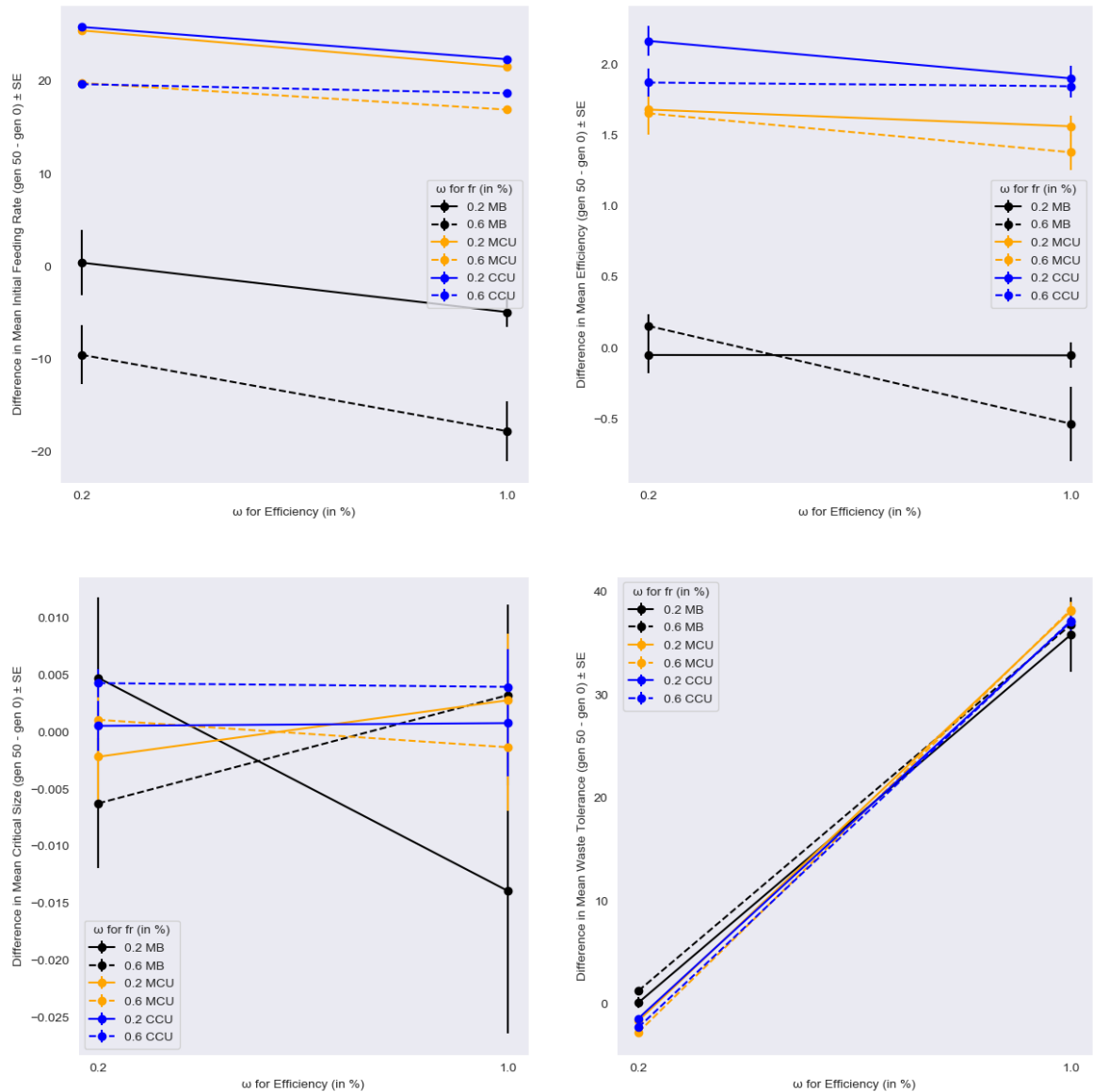

Figure 5.4: Effect of heritability in initial feeding rate (fr) and efficiency on mean trait values at generation 50.

Fig 5.5 shows that a decrease in heritability of critical size decreases mean initial feeding rate in both MCU and CCU cultures only when the heritability of initial feeding rate is high. Such effect is also seen at lower heritability of initial feeding rate only in CCU culture when (fig 5.5). In MCU culture, mean efficiency increases with an increase in the heritability of initial feeding rate at higher heritability of critical size. In contrast, there is no effect of heritability of initial feeding rate on mean efficiency when heritability of critical size lower in MCU culture. This pattern of mean efficiency with a heritability of these trait parameters is opposite in CCU culture. In MCU and CCU cultures, higher heritability of critical size causes no change in mean critical size over generations. Lower heritability of critical size causes a decrease in mean critical size in MCU and CCU cultures equally. This decrease shows interaction with the heritability of initial feeding rate, as higher heritability of initial feeding rate gives more decrease in mean critical size in both MCU and CCU cultures. There is no effect of the heritability of these trait parameters on mean waste tolerance.

Fig 5.6 shows overall no significant effect of heritability of critical size on the mean initial feeding rate. At higher heritability of efficiency, the mean initial feeding rate shows the difference between MCU and CCU culture. This difference between MCU and CCU cultures disappears at lower heritability of efficiency. Mean efficiency is higher for lower heritability of efficiency and higher heritability of critical size in MCU and CCU cultures. There is no effect of heritability of critical size on mean efficiency at higher heritability of efficiency in MCU culture. Mean efficiency between MCU and CCU culture is different when heritability of efficiency higher and that of critical size is lower. There is no effect of heritability of efficiency on mean critical size. Heritability of critical size only affects mean critIcal size when heritability of efficiency is high. Mean waste tolerance has a pattern similar to fig 5.4, showing that it is dependent on the heritability of efficiency.
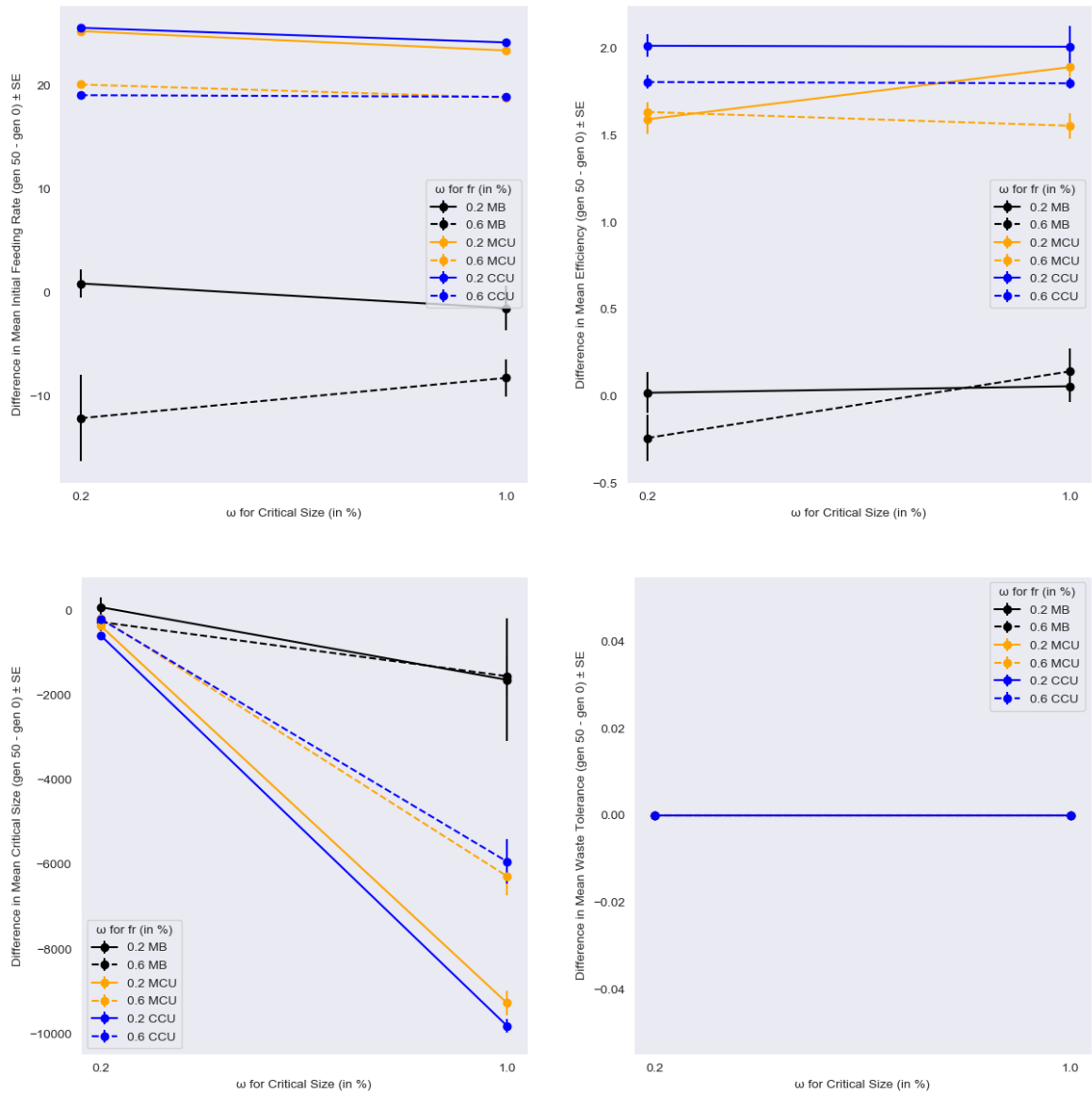
Figure 5.5: Effect of heritability in initial feeding rate (fr) and critical size on mean trait values at generation 50.
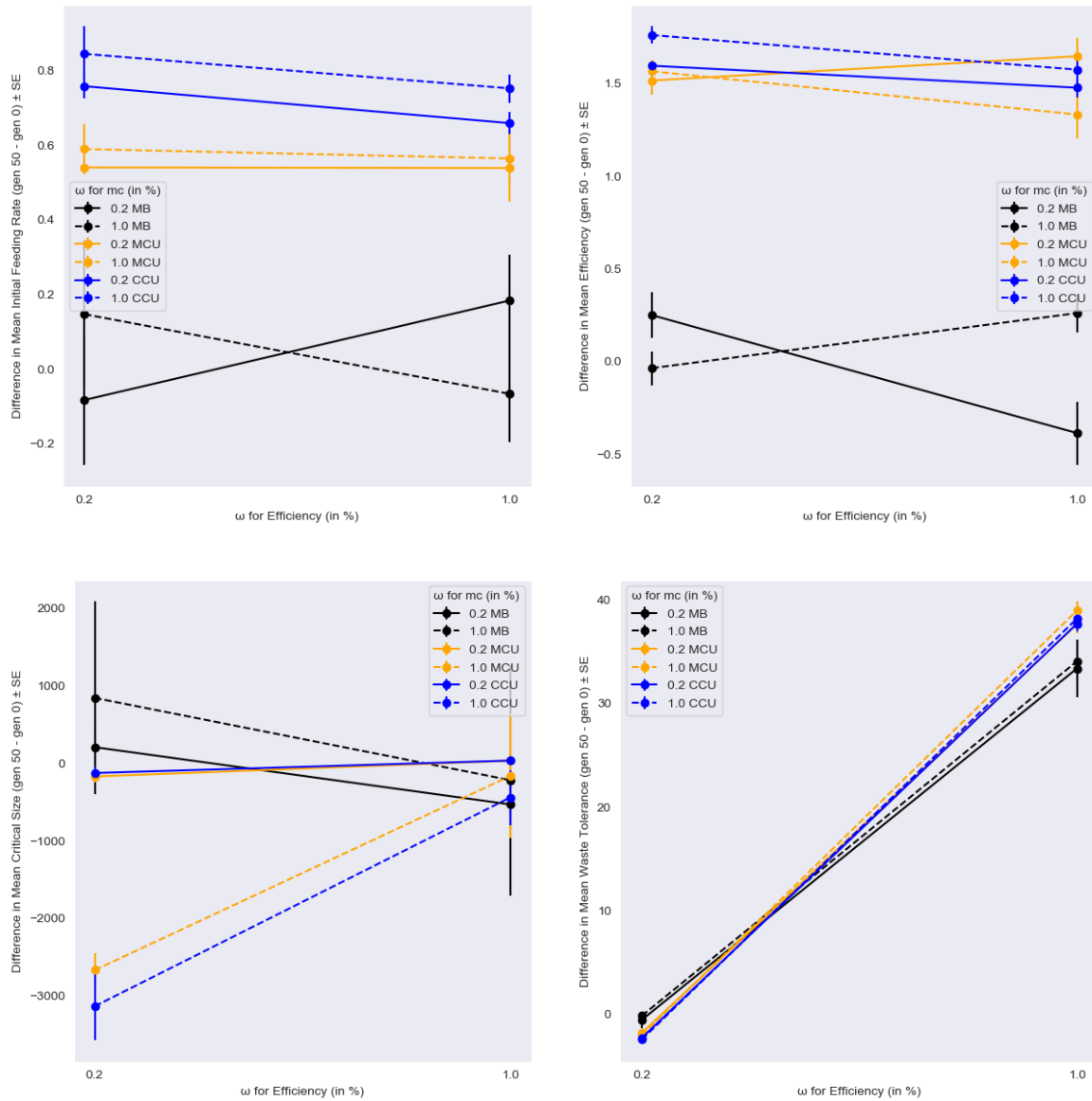
Figure 5.6: Effect of heritability in critical size (mc) and efficiency on mean trait values at generation 50.

Chapter 6

Introducing Correlations in Larval Trait Parameters

One of the interesting results that came out of studies on CU populations showed heritable polymorphism present in feeding rate and waste tolerance of these larvae (Daniel J. Borash et al., 1998). At high density, early eclosing larvae were faster feeding, less waste tolerant and faster developing, whereas late eclosing larvae were slower feeding, more waste tolerant and slower developing. Presence of such polymorphism most likely emerged from the way in which CU populations were maintained (Archana, 2009). Sarangi (2018) found a similar early-late pattern with body size and development time of larvae from MCU, CCU and LCU populations across high densities. It was also suggested that these populations might not show polymorphism with feeding rate and waste tolerance observed in CU populations. In this chapter, I have used the model to capture patterns in larval traits of early-late eclosing larvae across various larval densities. If the model is able to simulate results for mean body size distribution with development time similar to the experimental results, then it can be used to predict other mean larval trait value distributions with development time.

Larval trait parameters used in the model are considered to be independent of each other while assigning to each individual. This model can be useful to understand the distributions of these trait parameters and other life-history traits with development time after a few generations of selection in each density regime. It can also be used to

investigate how early eclosing and late eclosing larvae show differences in trait values at different densities.

6.1 Distribution of Laral Traits with Development Time

Simulations are run using the initial conditions for MB, MCU, CCU and MCU populations given in table A.3 at all four densities (replicates = 10, generation = 50). Results from these simulations are plotted for larval body size, final feeding rate (at critical size), efficiency, initial feeding rate, critical size and efficiency. Similar to previous simulations, time to reach critical size is taken as a proxy for development time (see fig 6.1 - 6.4). For each of the above traits, a scatter plot for mean trait value with development time is plotted ('x.estimator' method in 'seaborn' by Waskom et al. (2017), is used to plot mean trait values for each time point). These plots give a clear visualization of mean trait value distribution with development time. Mean trait values towards the right side of the x-axis represent late eclosing flies, whereas such values towards the left side of x-axis represent early eclosing flies.

In these plots, MB larvae take more to reach critical size compared to MCU, CCU and LCU larvae due to higher critical size, lesser efficiency and initial feeding rate. In MB culture (60 eggs / 6 ml), There is no pattern of body size, efficiency, initial feeding rate and waste tolerance with development time. Since the critical size is higher for larvae with higher development time, final feeding rate is also higher in them across all populations (see fig 6.1).

In MCU culture (600 eggs / 1.5 ml), results are similar to mean trait value distributions in MB culture except, decrease in mean body size of late eclosing ones in MCU population. Variation in mean body size is higher for both early and late-developing larvae for all populations in this culture (see fig 6.2).

In CCU culture (1200 eggs / 3 ml), mean body size and efficiency stays constant across development time, but the variation is very less for late developing larvae.

Initial feeding rate is slightly higher with a reduced variation for larvae with higher development time. Waste tolerance is higher with a decrease in variation for late developing larvae (see fig 6.3).

In LCU culture (1200 eggs / 6 ml), mean trait values are similar to those in CCU culture except, mean body size, which shows that late-developing larvae have similar body size as early-developing ones. These distributions form a U-shaped curve of mean trait values. For late-developing larvae in this culture, initial feeding rate is also lower, and variation in efficiency is higher across all populations. Mean waste tolerance distribution show late-developing larvae have higher waste tolerance which is more prominent in MCU and LCU populations (see fig 6.4).
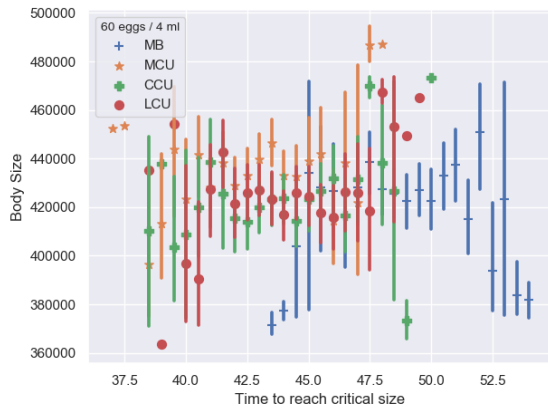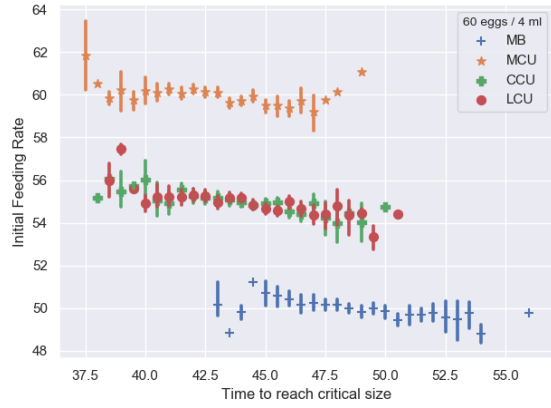
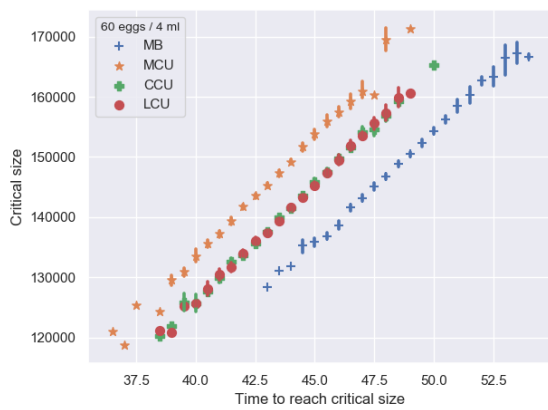(a) Mean body size distribution
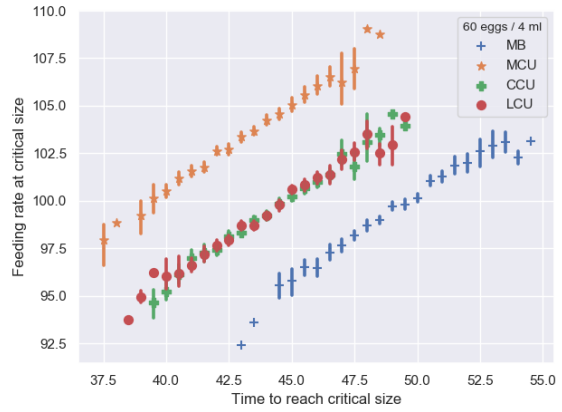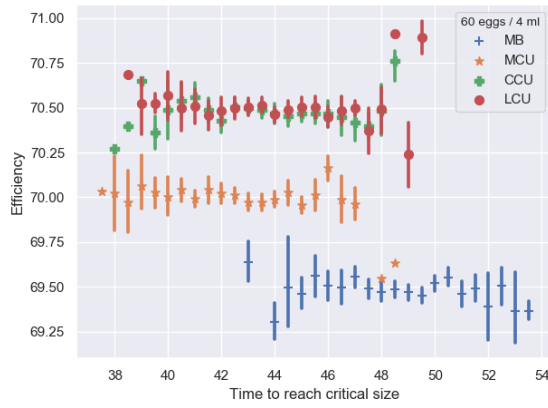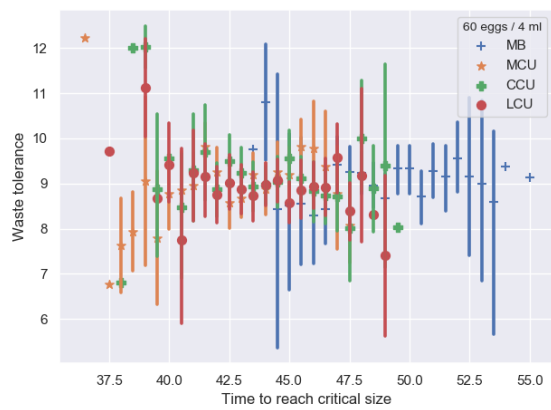
(b) Mean final feeding rate distribution

(c) Mean efficiency distribution

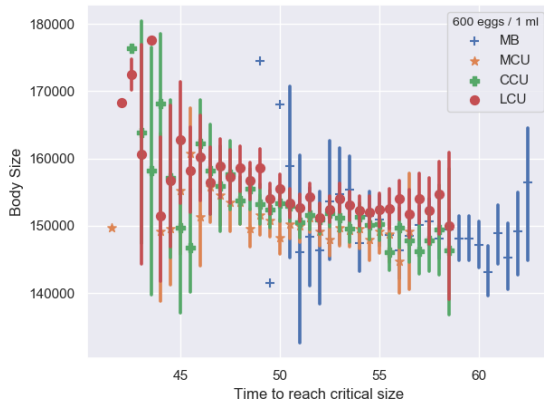(d) Mean initial feeding rate distribution

(e) Mean critical size distribution

(f) Mean waste tolerance distribution

Figure 6.1: Mean trait value distribution of MB, MCU, CCU and LCU populations in 60 eggs / 6 ml density at 50th generation

(a) Mean body size distribution

(b) Mean final feeding rate distribution

(c) Mean efficiency distribution

(d) Mean initial feeding rate distribution
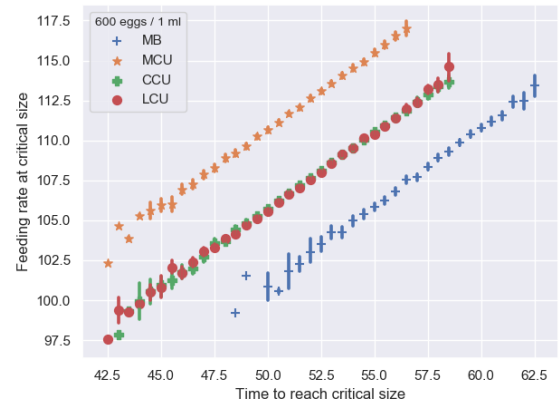
(e) Mean critical size distribution
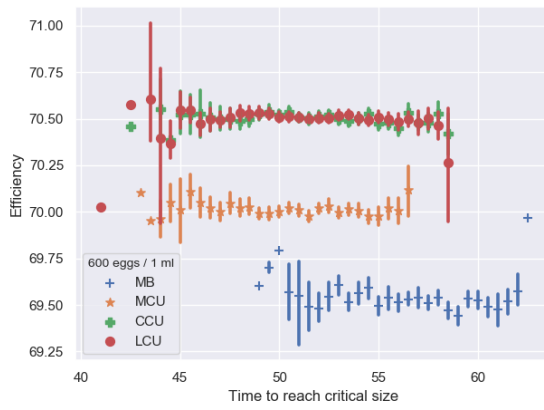
(f) Mean waste tolerance distribution

Figure 6.2: Mean trait value distribution of MB, MCU, CCU and LCU populations in 600 eggs / 1.5 ml density at 50th generation (errorbars represent 95% CI)
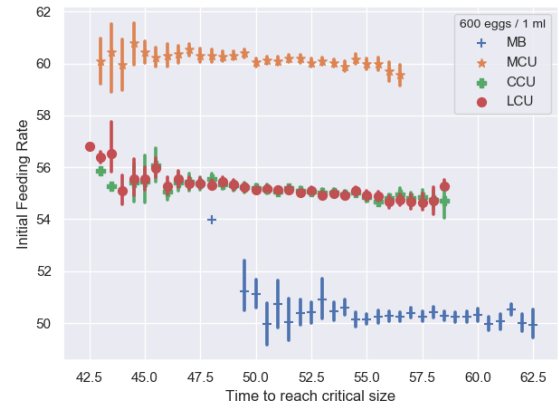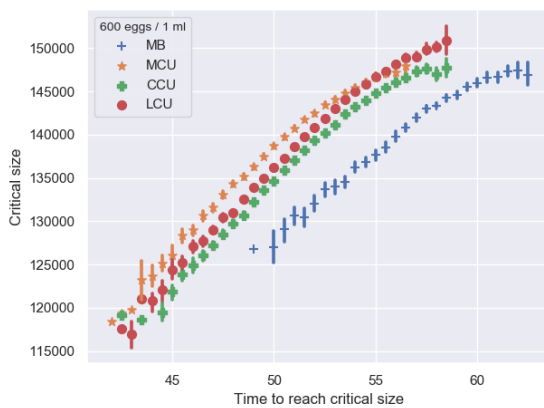
(a) Mean body size distribution

(b) Mean final feeding rate distribution

(c) Mean efficiency distribution

(d) Mean initial feeding rate distribution

(e) Mean critical size distribution

(f) Mean waste tolerance distribution

Figure 6.3: Mean trait value distribution of MB, MCU, CCU and LCU populations in 1200 eggs / 3 ml density at 50th generation (errorbars represent 95% CI)

(a) Mean body size distribution

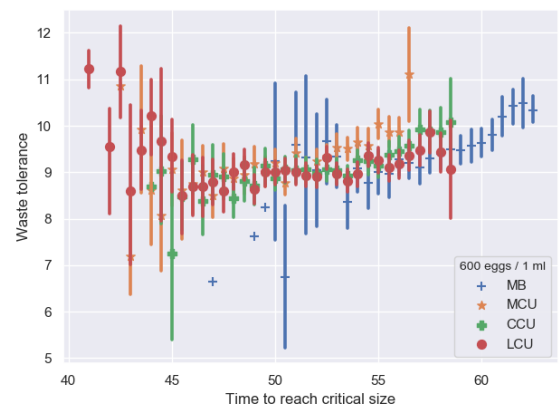(b) Mean final feeding rate distribution

(c) Mean efficiency distribution
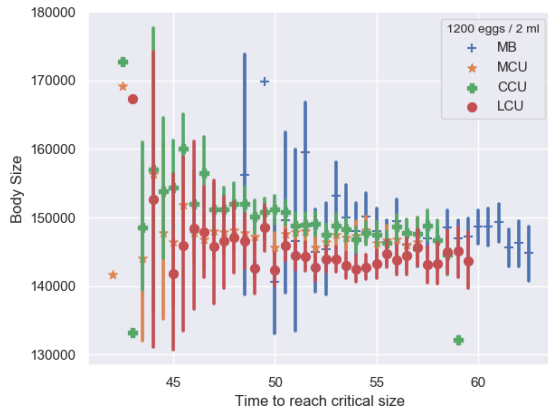
(d) Mean initial feeding rate distribution
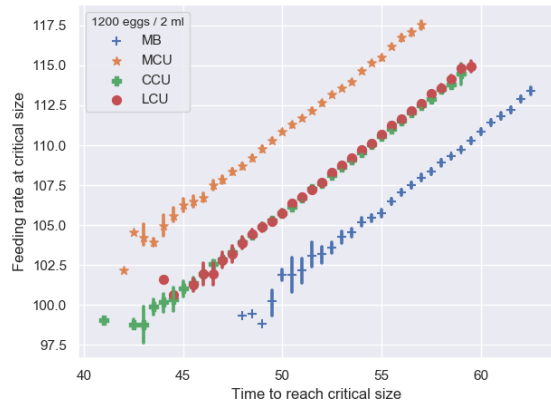
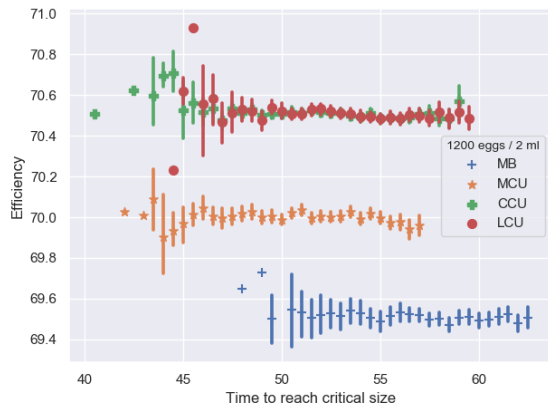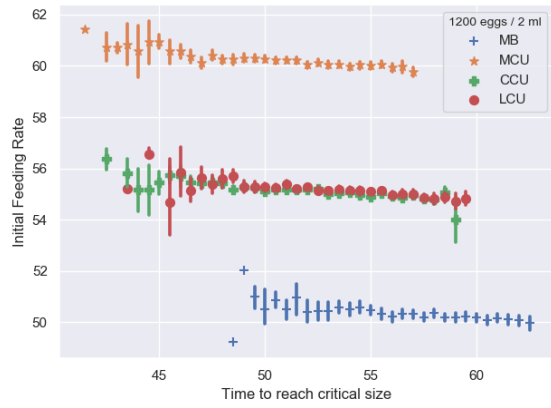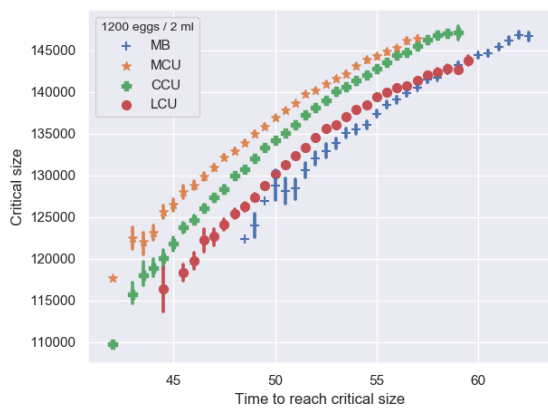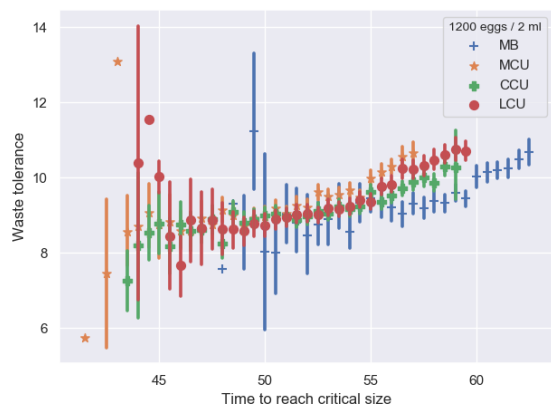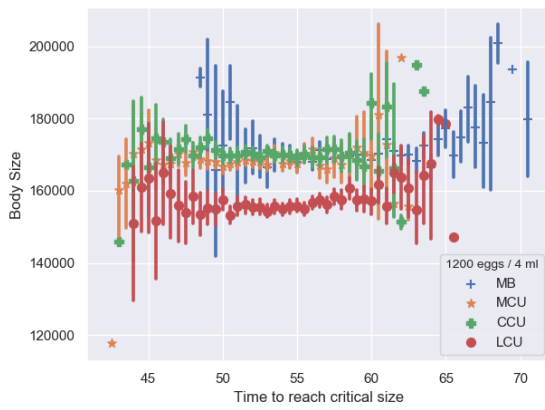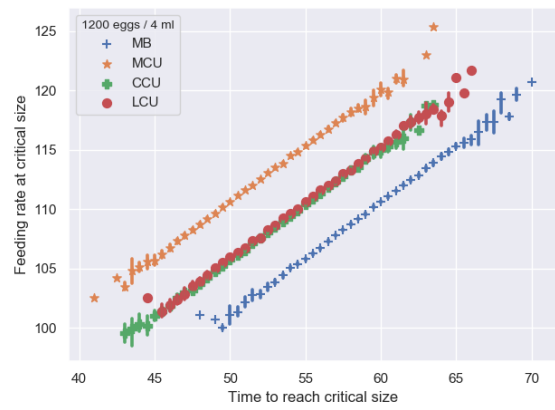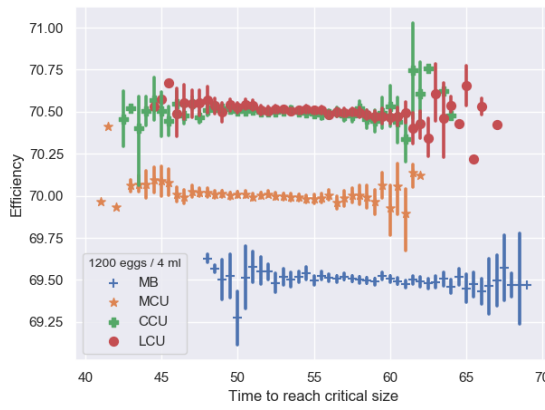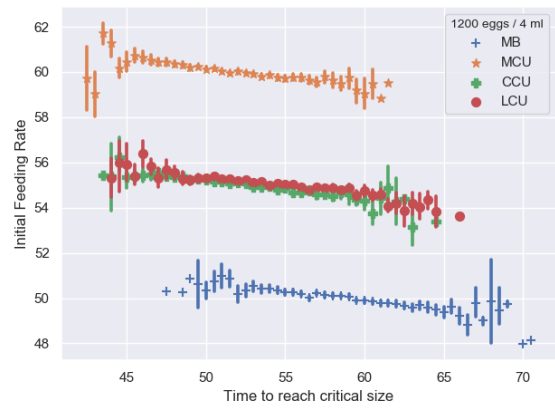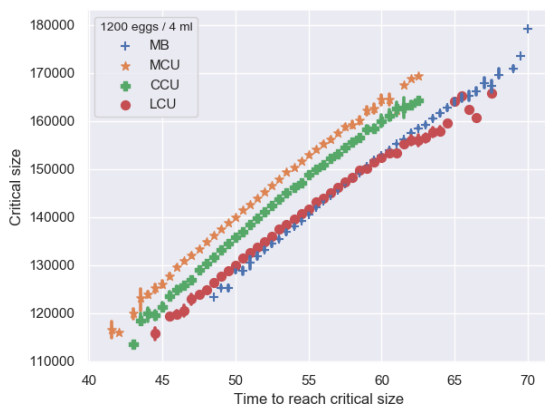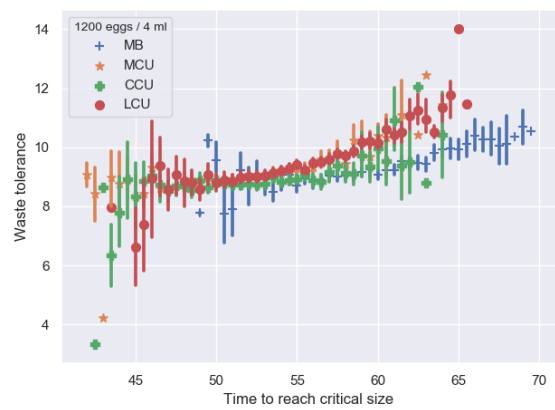(e) Mean critical size distribution

(f) Mean waste tolerance distribution

Figure 6.4: Mean trait value distribution of MB, MCU, CCU and LCU populations in 1200 eggs / 6 ml density at 50th generation (errorbars represent 95% CI)

6.2 Negative Correlation between Feeding Rate and Efficiency

From the mean trait distribution results, the effect of mean initial feeding rate distribution on body size distribution is clear (fig 6.3 and fig 6.4). According to the results mentioned above it is clear that if initial feeding rate is negatively correlated with development time, then the model would give a U-shaped body size distribution found in the empirical data of larval crowded populations (Sarangi, 2018). Based on the model of (Mueller & Barter, 2015), a negative correlation between initial feeding rate and efficiency in the model is introduced to understand how these correlations between larval competitive traits would affect other life-history traits. Here, efficiency to convert food into biomass is no longer a heritable trait in the model. Modifications were made such that efficiency is derived at each generation from the distribution of initial feeding rate. The relation between efficiency and intial feeding rate is given as:

$$\epsilon_i = K_\epsilon - x_6 * fr_i$$

Here, ϵ_i: efficiency of ith larva;

fr_i: initial feeding rate of ith larva;

x_6: scaling parameter;

K_ϵ: Maximum value of efficiency (constant).

Using this correlation, timeseries was run for initial conditions given in table A.1 and the similar simulations as mentioned in the previous section were performed (replicates = 5). The results show a negative correlation of initial feeding rate across all populations at all four densities (see fig 6.5). Due to the forced negative correlation between initial feeding rate and efficiency, the mean efficiency distribution shows that late-developing ones have higher efficiency (see fig 6.6). Fig 6.7 shows that final feeding rate is higher for late developing larvae even if there efficiency is higher and initial feeding rate is lower across all populations at all densities. Fig 6.8 was plotted with higher replicates (n=15) to obtain a clear visualization. Here MCU, CCU and LCU population have mean body size first decreasing then increasing along with all-time decreasing variation with respect to development time in MB and CCU culture. Such patterns are not evident in MB and LCU culture.
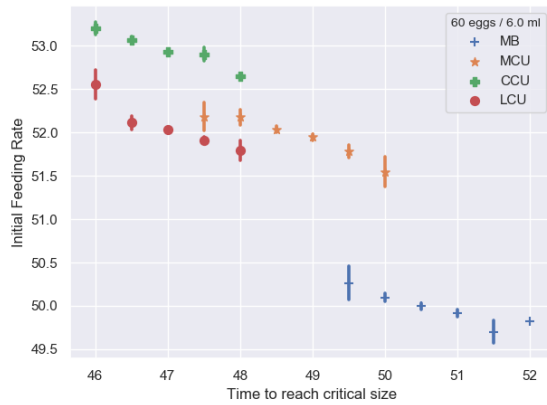
(a) 60 eggs / 6 ml
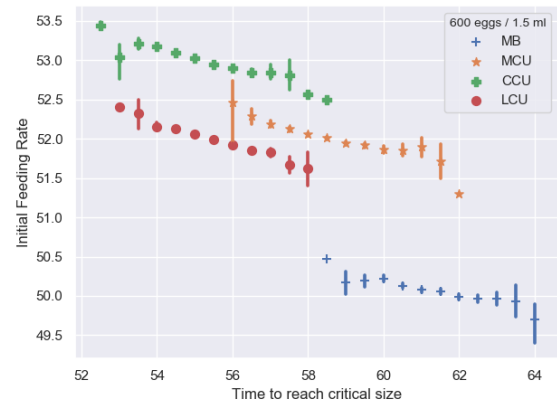
(b) 600 eggs / 1.5 ml
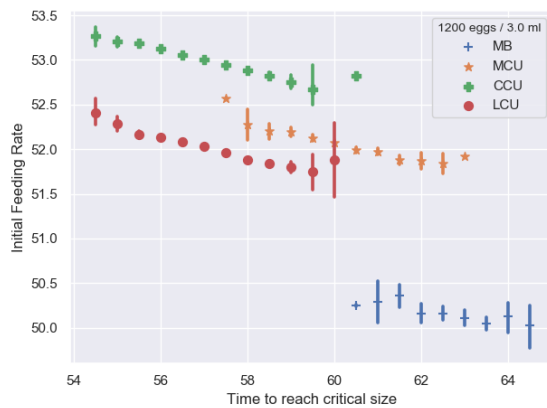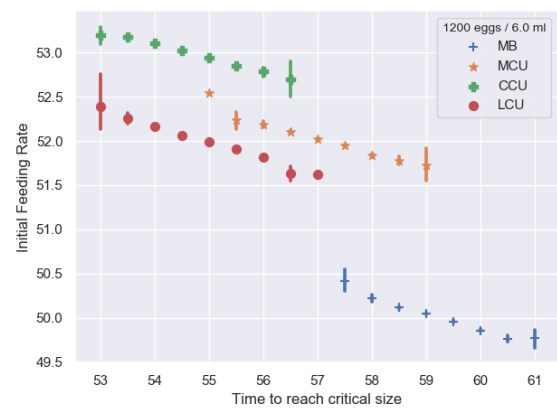
(c) 1200 eggs / 3 ml

(d) 1200 eggs / 6 ml

Figure 6.5: Mean initial feeding rate distribution of MB, MCU, CCU and LCU populations in (a) 60 eggs / 6 ml, (b) 600 eggs / 1.5 ml, (c) 1200 eggs / 3 ml and (d) 1200 eggs / 6 ml densities (errorbars represent 95% CI)
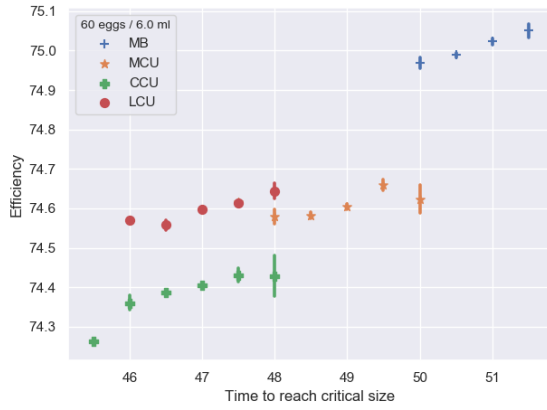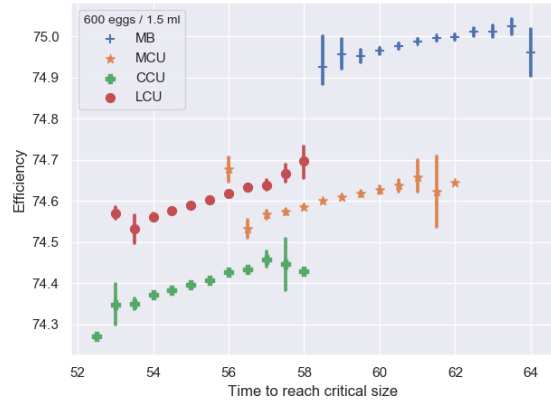
(a) 60 eggs / 6 ml
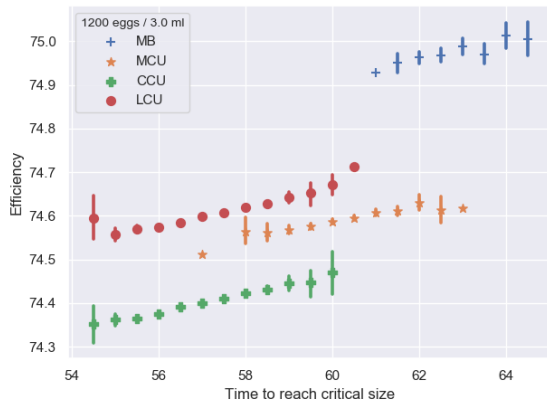
(b) 600 eggs / 1.5 ml
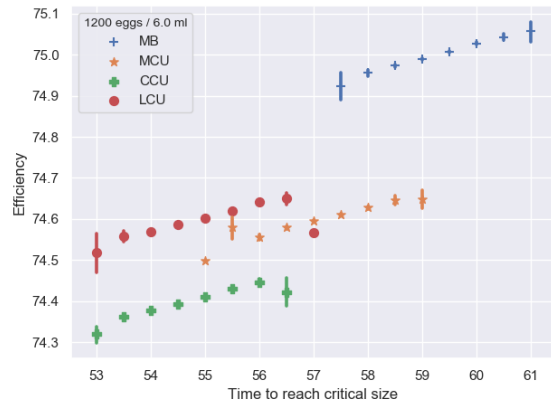
(c) 1200 eggs / 3 ml

(d) 1200 eggs / 6 ml

Figure 6.6: Mean efficiency distribution of MB, MCU, CCU and LCU populations in (a) 60 eggs / 6 ml, (b) 600 eggs / 1.5 ml, (c) 1200 eggs / 3 ml and (d) 1200 eggs / 6 ml densities (errorbars represent 95% CI)
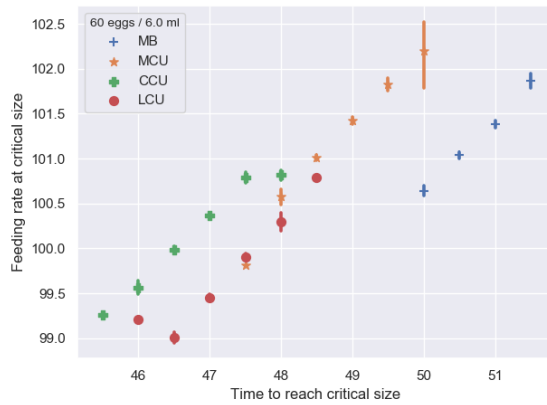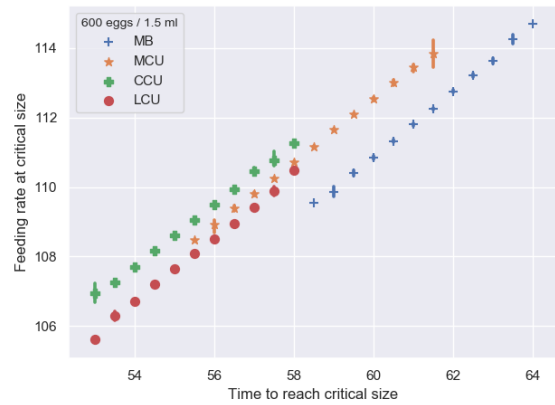
(a) 60 eggs / 6 ml
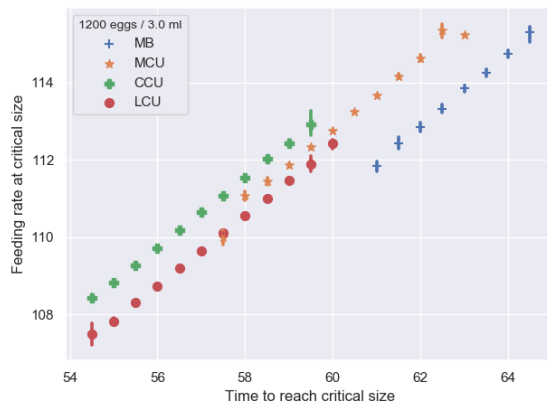
(b) 600 eggs / 1.5 ml
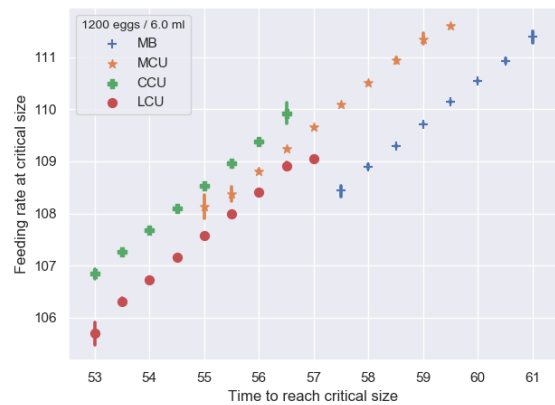
(c) 1200 eggs / 3 ml

(d) 1200 eggs / 6 ml

Figure 6.7: Mean feeding rate at critical size of MB, MCU, CCU and LCU populations in (a) 60 eggs / 6 ml, (b) 600 eggs / 1.5 ml, (c) 1200 eggs / 3 ml and (d) 1200 eggs / 6 ml densities (errorbars represent 95% CI)
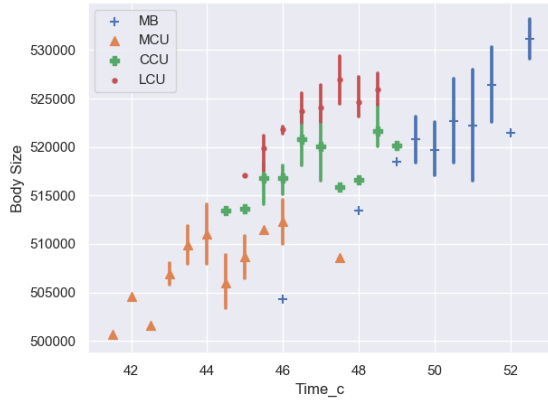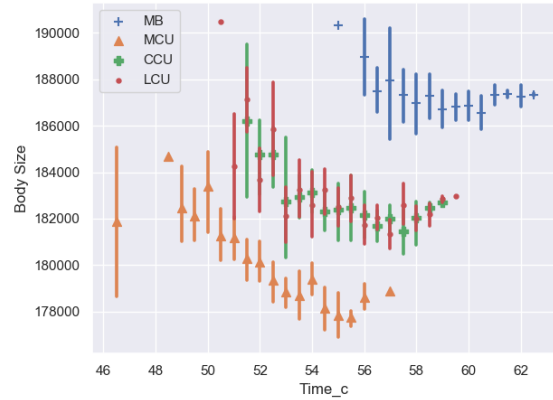
(a) 60 eggs / 6 ml
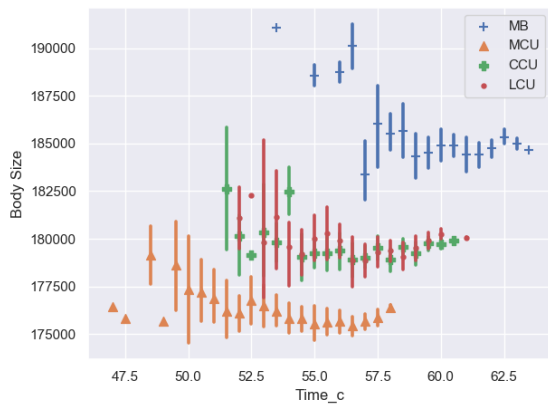
(b) 600 eggs / 1.5 ml
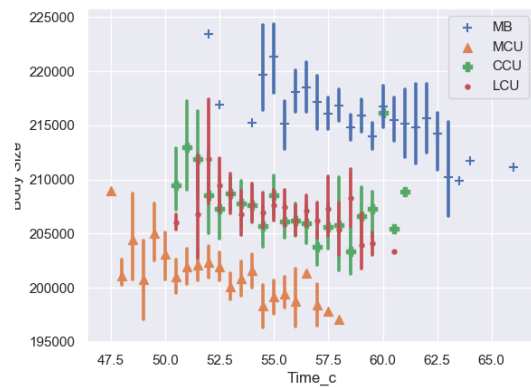
(c) 1200 eggs / 3 ml

(d) 1200 eggs / 6 ml

Figure 6.8: Mean efficiency distribution of MB, MCU, CCU and LCU populations in (a) 60 eggs / 6 ml, (b) 600 eggs / 1.5 ml, (c) 1200 eggs / 3 ml and (d) 1200 eggs / 6 ml densities (errorbars represent 95% CI)

Chapter 7

Discussion

Simulation results from the stage-structured individual-based model, which describe the ecological and evolutionary dynamics of *Drosophila melanogaster* populations adapted for various larval crowding conditions, are presented in this thesis.

In Larval stage simulations, it is evident that waste build-up in the feeding band is dependent on effective larval density (number of eggs per feeding band) along with total larval density (chapter 2). Both MCU and CCU cultures have the same larval density but still show different patterns of ecological dynamics inside the vial. Such a pattern might be one of the reasons why CCU populations evolved greater competitive ability through a set of larval traits different than in MCU populations. The model describes feeding band dynamics in detail which is limited by experimental setups. In these larval stage simulations, CCU and LCU cultures show similar feeding band dynamics with waste build-up and food quality decrease till most of the larvae reach critical size, even though their overall larval densities are different. This result suggests that effective larval density plays a vital role in determining fitness-related traits such as larval body size, development time and survivorship, as suggested by (Sarangi, 2018).

Further simulations performed on the larval stage showed the effect of larval trait parameters used in the model on fitness-related larval traits (chapter 3). These results show the density-dependent effect of initial feeding rate, efficiency and critical size on the larval body size, development time and survivorship. These larval trait

parameters interact differently in each culture due to differences in ecological dynamics. Such simulations help us to understand how a specific fitness-related trait value can be achieved through multiple sets of larval trait parameters (Sarangi et al., 2016).

One of the exciting results from the larval stage simulations shows that feeding rate at critical size is dependent on not only initial feeding rate but also efficiency, critical size and effective larval density (chapter 3). The feeding rate at critical size in the model being a linear function of time to reach critical size, is dependent on several trait parameters as well as ecological dynamics. Such effect of interactions between larval trait parameters and larval density on the final feeding rate also explains the density-dependent behaviour of feeding rate seen in the recent experimental studies. This model also explains how the recent empirical results showed MCU larvae with the highest feeding rate (post-critical) in culture vials (Sarangi, 2018).

Later chapters in the thesis are focused on the evolutionary side of the model (chapter 4 and 5). In these simulation results, larval trait parameters in the model seem to evolve aggressively in CCU culture than in MCU culture simultaneously. From these evolved trait parameters, the fitness-related traits are obtained at each density and are similar to experimental MB and MCU populations but not CCU populations. This possibly suggests that there might be other factors playing a role at the evolutionary stage other than just the ecological differences between MCU and CCU.

I have used the model to explain how evolutionary trajectories taken by these parameters are dependent on the variation which exists in the larval traits. In the model, the variation in each trait comes from initial standing variation and heritability. Simulations results from chapter 5 suggest that initial standing variation in critical size can affect the evolutionary trajectory of efficiency such that the efficiency of MCU population is different from CCU population. Initial standing variation also seems to positively correlate with maximum mean trait value a trait can attain after several generations of selection. Heritability of larval traits in the model also seems to play a critical role in determining which trait evolves for greater competitive ability. These results show that heritability of one trait can affect the mean trait value of

another trait in several cases of MCU and CCU populations differently. For example, the evolution of lesser critical size occurs only when heritability of efficiency is high along with lesser heritability of critical size in both MCU and CCU populations.

The effect of heritability and intial standing variation on the evolution of larval traits is more prominently seen in CCU populations than MCU populations. This could be due to extreme effective larval density pf CCU populations as compared to MCU populations. In the model, MCU and CCU populations can have different trait values after several generations of selection if heritability and initial standing variation are calibrated enough. Results from these simulations suggest that ecological dynamics is just one part of the story where MCU and CCU populations differ and that these sources of variation in trait values might be playing a much more significant role than previously thought. This model suggests that further experimental studies on the heritability of larval traits are required to understand the differences competitive ability of MCU and CCU populations.

The model also used in the investigation of the larval trait distribution with development time. The simulations for these trait distributions are aimed at exploring the patterns of trait polymorphism present in early-late eclosing larvae (chapter 6). The results show that early eclosing larvae have a lesser final feeding rate and critical size at different densities. Late eclosing larvae seem to show higher waste tolerant, and such polymorphism is maintained in all four populations without displaying overall change in mean waste tolerance. First set of simulations involved larval trait parameters which were independent of each other but still showed correlation patterns in trait distribution with development time. The simulations at LCU density give U-shaped distribution of body size observed in experimental populations (Sarangi, 2018). From the patterns of initial feeding rate, it was inferred that lower initial feeding rate of late eclosing larvae is responsible for their higher body size. Thus, the second set of simulations are performed with a negative correlation in mind to explain the presence of U-shaped body size distribution in MCU and CCU density as well. These preliminary simulation results suggest that all larval trait parameters exhibit polymorphism seen in early-late eclosing larvae, some of them might be

correlated with each other, and they are responsible for determining U-shaped body size distribution in CCU and LCU populations across MCU, CCU and LCU densities.

The stage-structured individual-based model presented here, acts as a computational tool used for a better understanding of ecological and evolutionary dynamics of MB, MCU, CCU and LCU populations. The model simulates results which are mostly in correlation with empirical data. Since the model explores, various factors such as ecological details, initial standing variation, heritability, and polymorphism play a role in density-dependent selection studies so far. These factors are responsible for determining evolutionary routes taken to achieve greater competitive ability. The model is very flexible as several variables have been taken into account, and these variables can be refined or modified for the different crowded population. The sexually dimorphic traits considered in the models can also be studied in future to investigate how sex might play a role in different larval crowded conditions. The model is based on basic inheritance rules which can also be modified for a better realistic approach. It is hoped that outcomes from this work will enable a better understanding of adaptation to crowding environment in not only *Drosophila* but other holometabolous insects as well.

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Appendix A

Supplementary Data

A.1   Code

All code is written in Python 3.7 ("Python Software Foundation", www.python.org).

The entire code is on the following Github repository:

https://github.com/ElY0da/Thesis_code.git

Figure A.1: QR code for Github repository containing all codes

A.2 Tables

No.	parameters	Symbol	Value
1.	Larval food (1.5 ml)	$food$	1.85e8
2.	initial body size (t=0)	$S_i(0)$	3.0
3.	Proportion of waste diffusion	k_d	0.12
4.	Proportion of waste diffusion in LCU vial	k_d	0.18
5.	Feeding band size	$fband$	8.9e9
6.	Diffusion band size	$dband$	8.9e9
7.	LCU feeding band size	$fband$	7.4e9
8.	LCU diffusion band size	$dband$	7.4e9
9.	Volume of food eaten / bite (pre-cirtical)	$V_f(pre)$	1.0
10.	Adult nutrition	Nut	1.49
11.	Maximum value of efficiency	K_ϵ	85.0

Table A.1: Values of constant parameters used for initiating the model

No.	Larval Trait	Symbol	Distribution
1.	Initial feeding rate	fr_i	$N(50.0, 1.0)$
2.	Critical size	sc_i	$N(1.5e5, 7.5e3)$
3.	Efficiency	ϵ_i	$N(69.5, 0.2)$
4.	Waste tolerance	u_i	$N(9.0, 1.0)$

Table A.2: Distributions and values of larval trait parameters used for initiating the model

No.	Larval Trait	MB	MCU	CCU	LCU
1.	Initial feeding rate	50	60	55	55
2.	Efficiency	69.5	70.0	70.5	70.5
3.	Critical size	1.5e5	1.45	1.41	1.41
4.	Waste tolerance	9.0	9.0	9.0	9.0

Table A.3: Mean values of trait parameters used for initiating simulations of mean trait value distribution (no correlation)

No.	Larval Trait	MB	MCU	CCU	LCU
1.	Initial feeding rate	50	56	52	53
3.	Critical size	1.68e5	1.57	1.5	1.52
4.	Waste tolerance	7.0	7.0	7.0	7.0

Table A.4: Mean values of trait parameters used for initiating simulations of mean trait value distribution (negative correlation)

No.	Parameter	Value
1.	x_1	0.017
2.	x_2	1e4
3.	x_3	7e-6
4.	x_4	15.0
5.	x_5	1.0
6.	x_6	0.2

Table A.5: Values of scaling parameters used in the model

A.3 Graphs

The Supplementary graphs for results in Chapter 3, 5 and 6 are on the following Github repository: https://github.com/ElY0da/Thesis_results.git

Figure A.2: QR code for Github repository containing all the results