Ecological and Evolutionary Dynamics of *Drosophila melanogaster* Populations Adapted for Larval Crowding

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Introduction

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 (ref), diffusion of waste in the food below (ref), total food amount (ref). 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 waste excreted, development time (ref). 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 Vial in Drosophila Cultures

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 (ref), and this accessible part of the food is referred as the feeding band. 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. The

growth of larvae in the model 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.

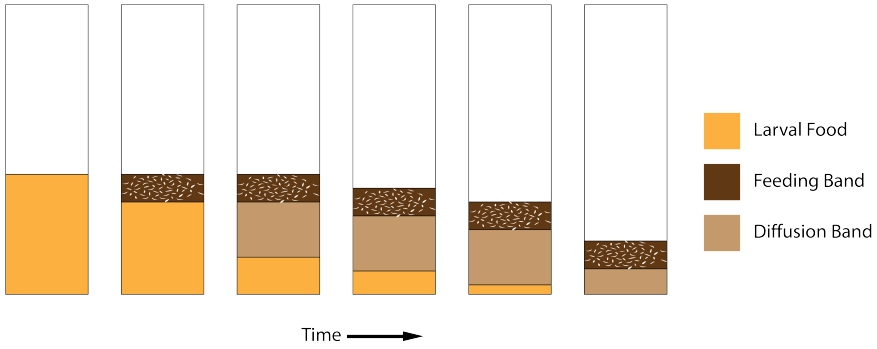


Figure 2.1: Ecological dynamics in a vial during larval feeding

2.2 Larval Stage Model

Each individual egg is assigned larval trait parameters from respective distributions with certain mean and variation given in table B.1. 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. (ref)

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:

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

Here,

fr_i: initial feeding rate of i^{th} larva; x_1: scaling parameter,

t: given time step; $Fr_i(t)$: Feeding rate at time t

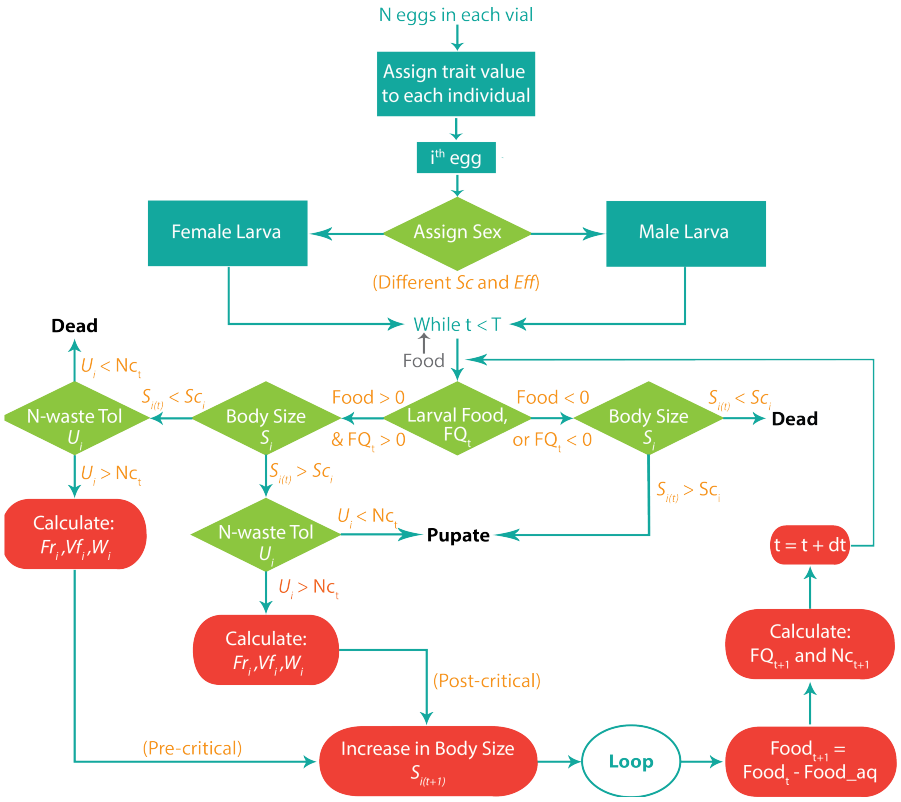


Figure 2.2: Flowchart of the larval stage in the model

Feeding rate stays constant During post-critical stage. 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 i^{th} larvae,

$FQ_{fb}(t)$: 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 B.1, table B.2 and table B.3. These values were obtianed by calibrating survivorship, body size and development time results similar to the empirical results in various larval densities(ref).

2.3 Simulations for Feeding Band Dynamics

Simulations are performed for trait values in table B.1 to observe the waste build up dynamics in a food vial with different larval densities during larval feeding. 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.

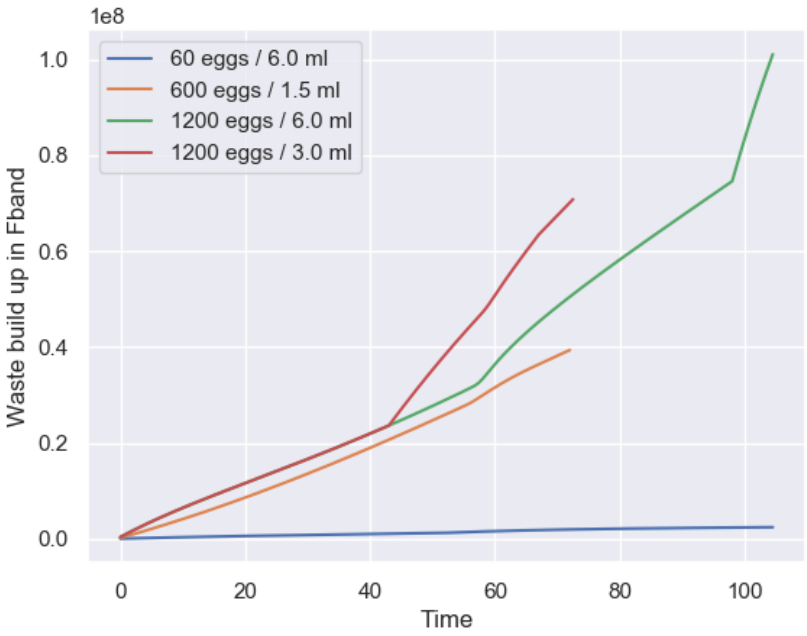


Figure 2.3: Waste build up in the feeding band

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 food below feeding band in CCU culture where waste can diffuse. After approx. 40^{th} 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 60^{th} time step. This is due to the food is still available below the feeding band. At approx. 100^{th} 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.

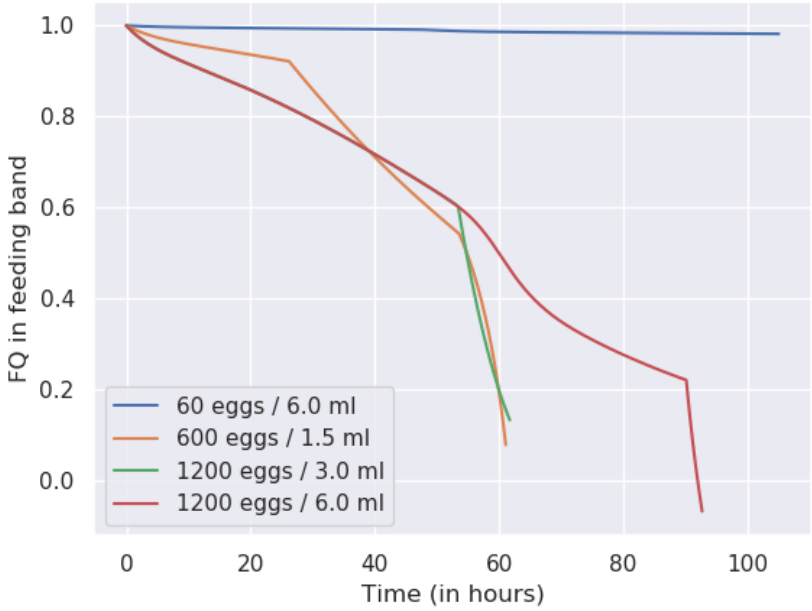


Figure 2.4: Change in the food quality of feeding band

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. it can be interpreted that body size increment between 40^{th} and 60^{th} time step is completely different for MCU and CCU cultures even though there larval density is equal. In LCU culture, decrease in food quality is simlar to CCU culture till 60^{th} time step, but later follows the same waste build-up dynamics as explained above.

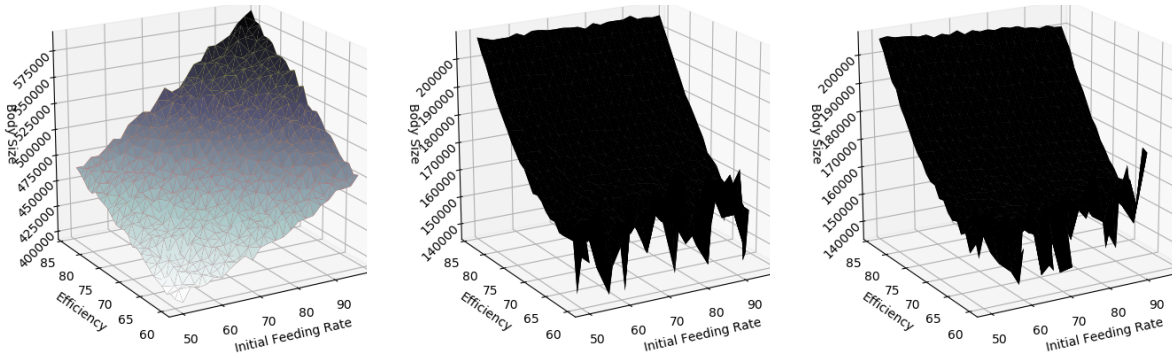
Chapter 3

Interplay between Larval Trait Parameters

In the base model of larval stage, trait parameters used are initial feeding rate, efficiency, critical size and waste tolerance. These parameters can not be measured directly via experimental approaches, but their effect on other larval traits such as body size, feeding rate at the third instar, development time can be measured easily. 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 feeding rate in the model stays constant after reaching critical size, it can be taken as proxy for feeding rate at the third instar stage.

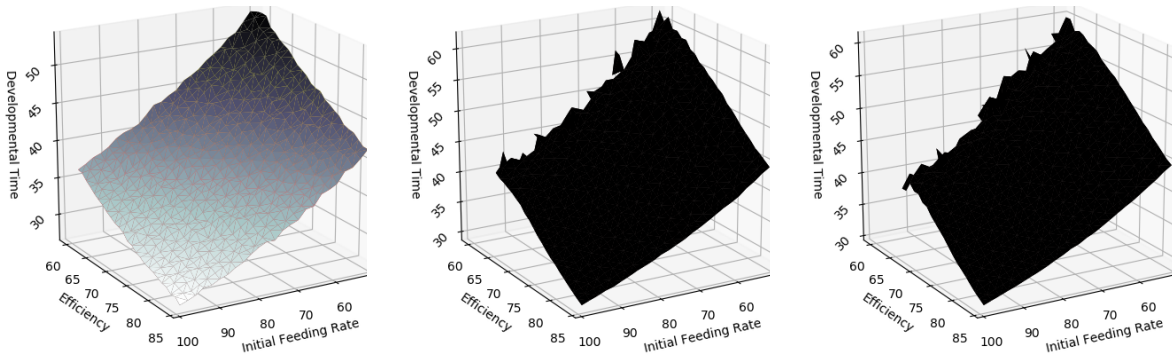
3.1 Initial Feeding Rate and Efficiency

In MB culture (low density), fig **??** shows having higher efficiency as well as higher initial feeding rate gives higher larval body size, but lower time to reach critical size. Feeding rate at critical size is dependent on time taken to reach critical size which is dependent on body size increment at each time step. This body size increment is proportional to the current feeding rate and efficiency. Thus, efficiency and initial feeding rate both affect the feeding rate shown at the critical size. Having lower efficiency and higher initial feeding rate tends to give higher feeding rate at critical size in MB culture. Survivorship does not show any pattern at low density, since most of the larvae are competing very less and are able to survive easily.



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

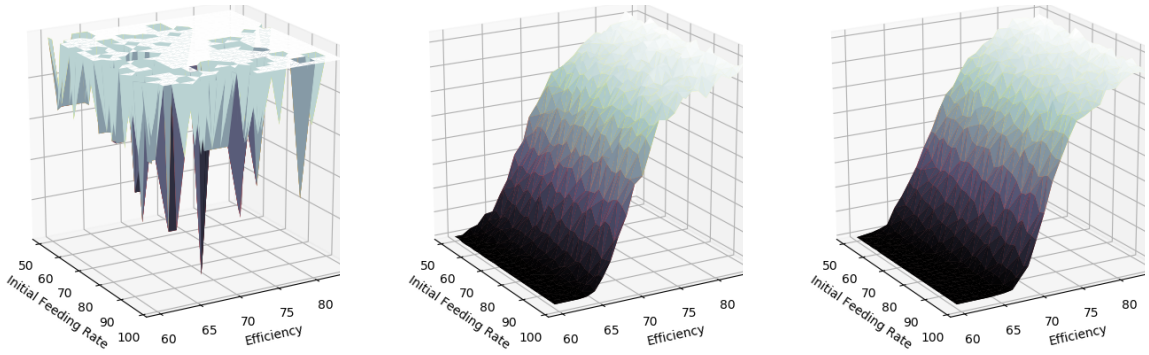
Figure 3.1: Effect of initial feeding rate and efficiency on body size at different larval densities



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

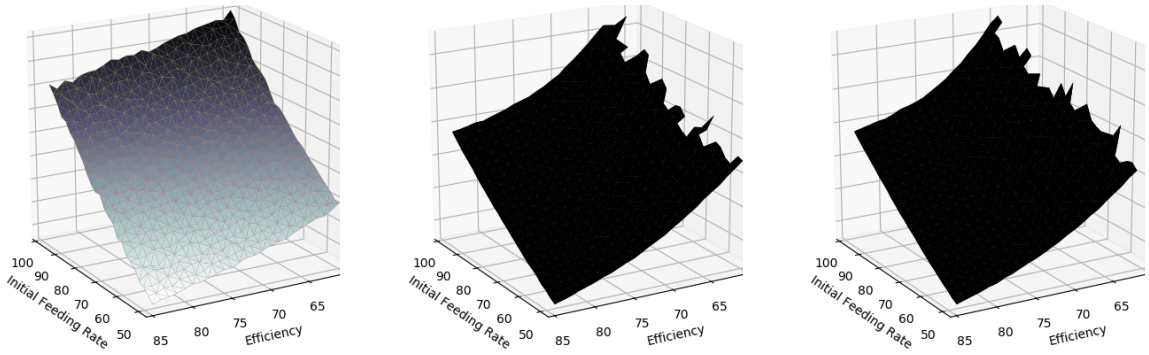
Figure 3.2: Effect of initial feeding rate and efficiency on developmental time

In MCU and CCU cultures (high densities), fig 3.5 and fig 3.6 show that time to reach critical size show similar pattern as seen in MB culture with varying efficiency and initial feeding rate. The maxima possible is higher in high density cultures than maxima possible in low density culture, showing that it takes more time to reach given critical size at high densities than at low density with same efficiency and initial feeding rate values. Feeding rate shown at critical size also shows similar pattern as seen in MB culture but with higher maxima reached with same parameter ranges. This suggests feeding rate shown at critical size is also a density dependent trait.The complete white pixels in all heatmaps (fig 3.5 and fig 3.6) are the values where none of the larvae survived, so the trait could not be measured and are to be excluded.



(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

At high densities, especially at MCU density, body size and survivorship are not affected by initial feeding rate, unlike at low density. Food acquired while having either higher or lesser initial feeding rate, remains almost the same. This is due to the decrease in food quality is higher for higher initial feeding rate. Thus, overall body size increment which is majorly determined by food quality at high densities, is approximately same in both cases i.e. larval growth with both higher and lower initial feeding rate. Survivorship also shows similar pattern as body size for these two parameters, since it is determined by whether critical size is reached or not.

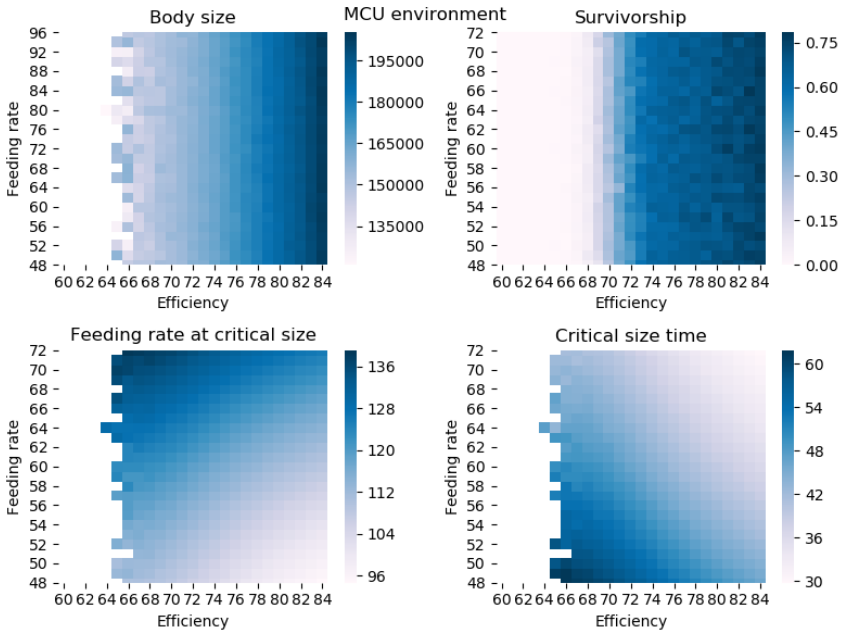


Figure 3.5: Effect of initial feeding rate and efficiency on larval traits in MCU culture

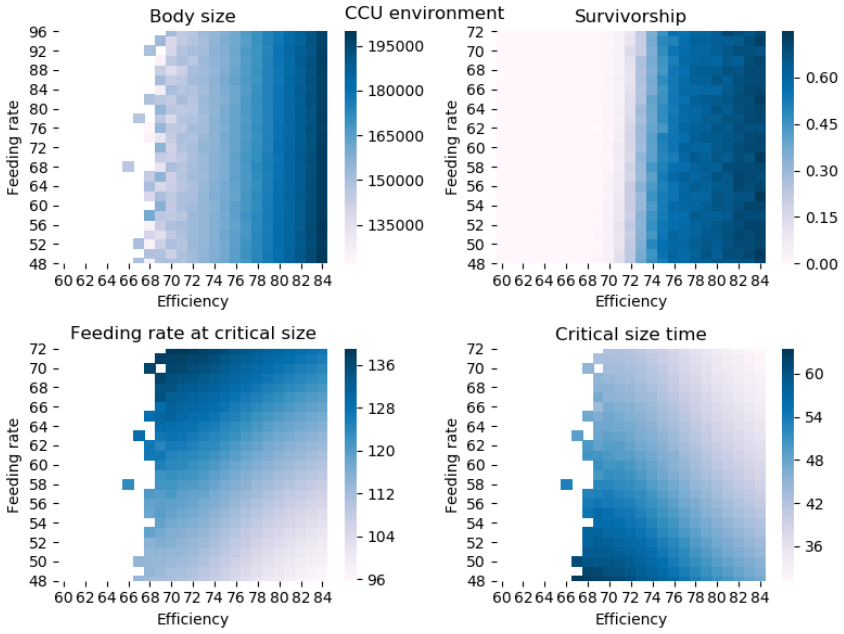


Figure 3.6: Effect of initial feeding rate and efficiency on larval traits in CCU culture

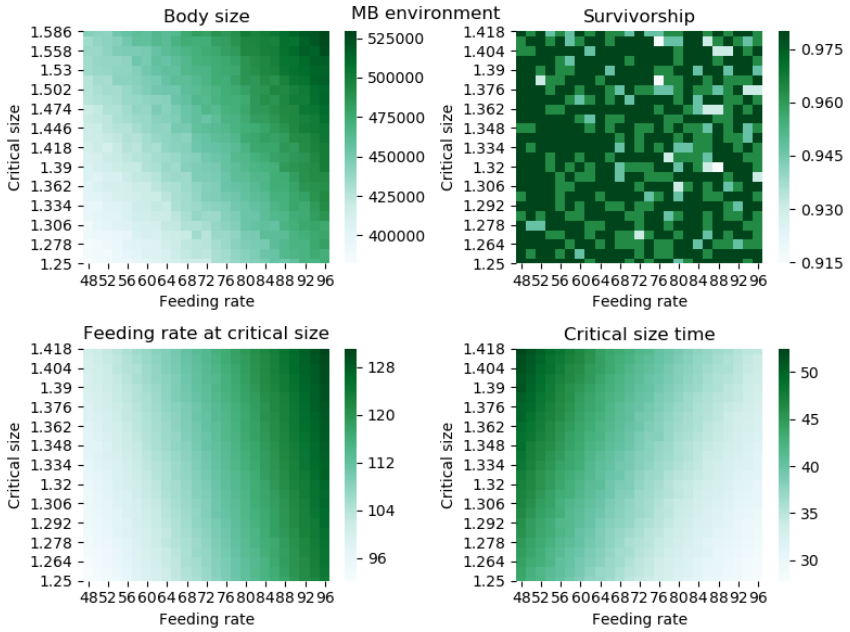


Figure 3.7: Effect of initial feeding rate and critical size on larval traits in MB culture

3.2 Initial Feeding Rate and Critical Size

In MB culture, fig 3.7 shows having higher critical size and higher initial feeding rate leads to higher larval body size. Lower critical size and higher initial feeding rate is beneficial in having lower time to reach critical size. Feeding rate at critical size is majorly determined by initial feeding rate, while having higher critical size gives slightly higher feeding rate, since time to reach critical size is more. Survivorship is not affected by either initial feeding rate or critical size at low density, since competition for food is minimal.

In MCU culture fig 3.8, body size is not affected by either critical size or initial feeding rate and post-critical growth is very less. Time to reach critical size and feeding rate at critical size show similar pattern as shown in MB culture with higher maxima for same values. Survivorship is only affected by critical size and initial feeding rate has no effect on survivoship at MCU density. Lower critical size shows trend for higher survivoship, since larvae are able to pupate in lesser time before food quality decreases drastically.

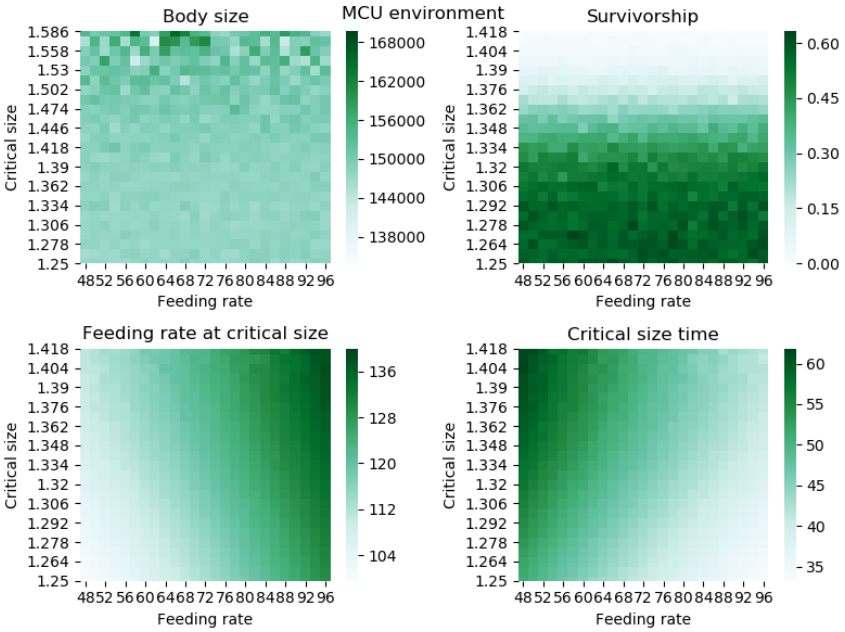


Figure 3.8: Effect of initial feeding rate and critical size on larval traits in MCU culture

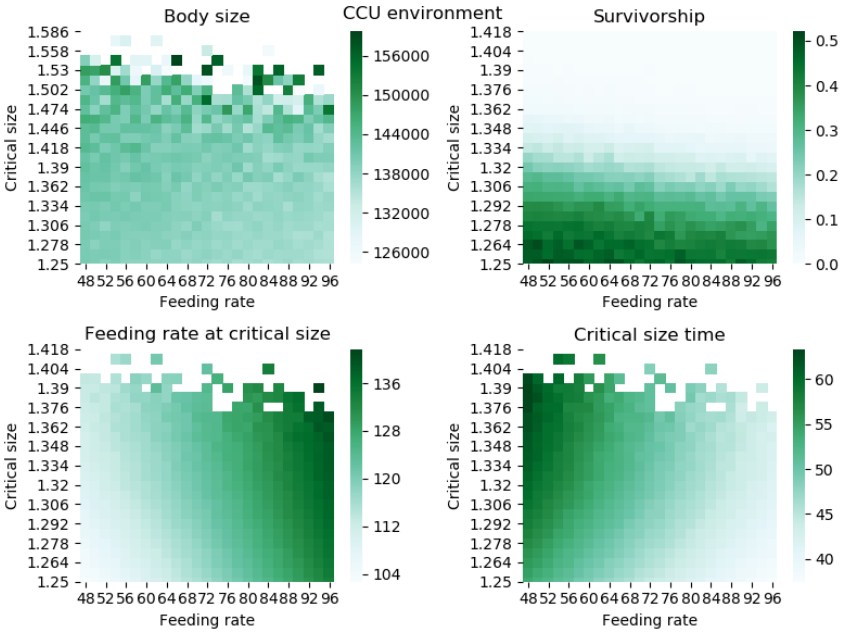


Figure 3.9: Effect of initial feeding rate and critical size on larval traits in CCU culture

In CCU culture, fig 3.9, patterns of body size, time to reach critical size and feeding rate at critical size similar to the ones in MCU culture. Survivorship shows small

effect of initial feeding rate as well along with critical size. Having lesser average initial feeding rate in the population leads to slower urea build up and decrease in food quality of the feeding band, thus larvae with higher critical size are able to survive.

3.3 Critical Size and Efficiency

In MB culture, fig 3.10, shows increase in body size with increase in critical size and efficiency. Feeding rate at critical size and time to reach critical size both increase with increasing critical size and decreasing efficiency. Higher efficiency leades to decrease in critical size thus feeding rate shown at critical size reaches lesser maxima. Survivorship shiws no effect of these parameters at low density.

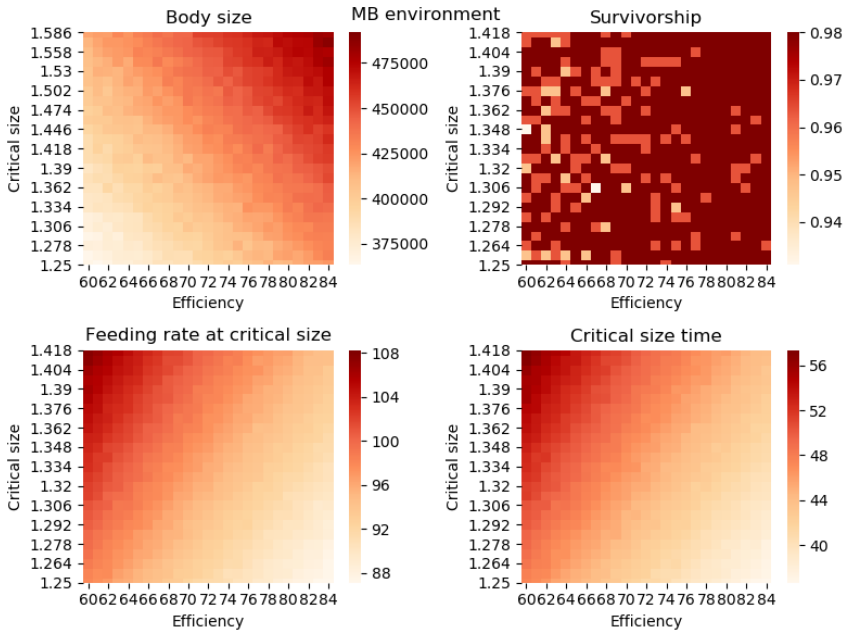


Figure 3.10: Effect of critical size and efficiency on larval traits in MB culture

In MCU and CCU culture, fig 3.11 and fig 3.12, all the larval traits show similar pattern as in MB density with critical size and efficiency. The maxima for feeding rate at critical size and time to reach critical size is higher compared to the ones at MB density. Body size and survivoship maxima are lesser at high density.

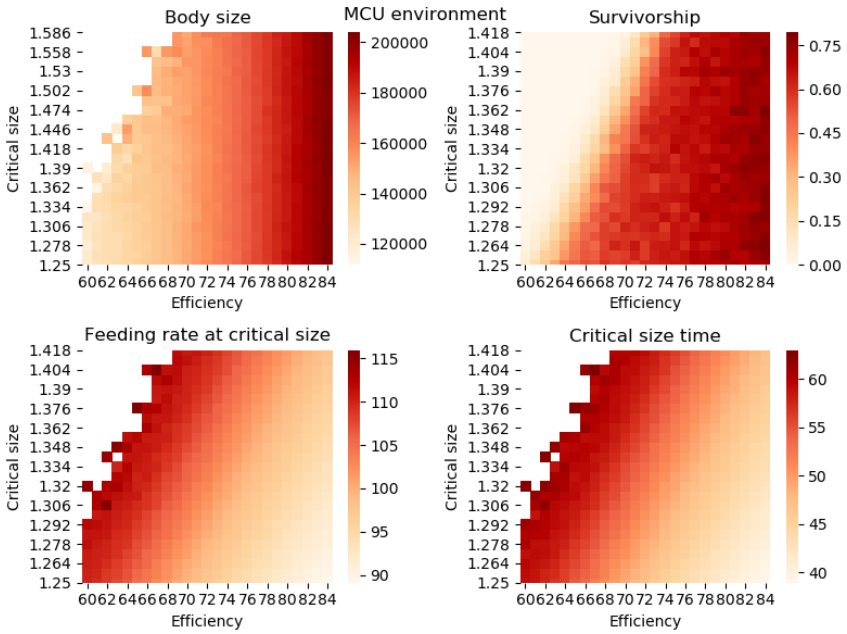


Figure 3.11: Effect of critical size and efficiency on larval traits in MCU culture

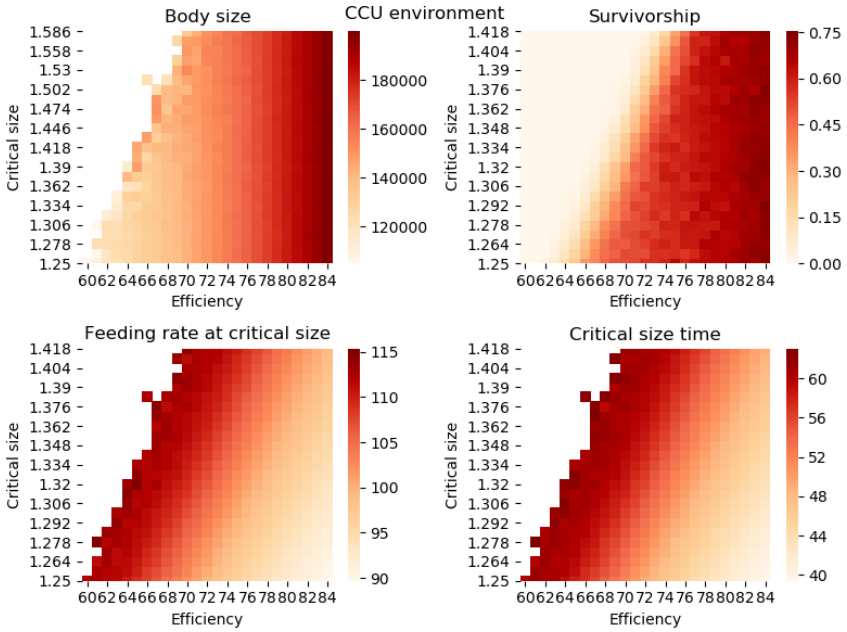


Figure 3.12: Effect of critical size and efficiency on larval traits in CCU culture

Appendix A

Code

Appendix B

Tables

This appendix contains values of all paramters used in the simulations.

B.1 Larval Trait Parameters

No.	Larval Trait	Symbol	Distribution
1.	Initial feeding rate		$N(,)$
2.	Critical size		$N(,)$
3.	Efficiency		$N(,)$
4.	Waste tolerance		$N(,)$

Table B.1: Distributions and values of larval trait parameters used for initiating the model

B.2 Scaling Parameters

No.	Paramter	Value
1.	x_1	0.017
2.	x_2	1e4

Table B.2: Values of scaling parameters used in the model

B.3 Other Larval Parameters

No.	Paramter	Symbol	Value
1.	Larval food (1.5 ml)	$food$	1.85e8
2.	Inital body size (t=0)	$S_i(0)$	3.0
3.	Proportion of waste diffusion	k_d	0.12
4.	Feeding band size	$fband$	7.4e9
5.	Diffusion band size	$dband$	7.4e9
6.	Volume of food (pre-cirtical)	$V_f(pre)$	1.0

Table B.3: Values of larval parameters used for initiating the model