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Control system for rotifer production

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Problem Description

Rotifers are used extensively as start feed for many cultured marine fish species and commercial size hatcheries require stable daily supply of high quality rotifers of substantial volumes. Considering the widespread success of control engineering principles in other industries, this project aims to explore the potential of employing such methods to large scale rotifer production systems in order to enhance production quality and reliability. The project should include the following tasks:

- Theoretical study of rotifer biology and state of the art rotifer production systems
- Identify state variables, inputs, outputs and disturbances and describe process dynamics in terms of mathematical relations
- Synthesize and investigate possible control strategies through simulation studies and experiments
- Documentation and conclusion

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Supervisor: Jo Arve Alfredsen, ITK

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Abstract

Rotifers are used extensively as start feed for many cultured marine fish species and commercial size hatcheries require stable daily supply of high quality rotifers of substantial volumes. This is often done relying on some employees whose knowledge and experience of the process and procedures ensures a stable production.

Control techniques have been used in many other industries during many years improving the quality, reliability, predictability and reducing the costs of the production. However, control engineering is not as widely used as in other industries yet and this is the objective of this thesis, study the possibilities of using such techniques in the area of rotifer production at large scale. The benefits of their application will be an increment in the quality and predictability of the production as it becomes less dependant on the experience of people, but on their experience combined with monitoring and control techniques that will maintain the best conditions possible for the cultivation all the time. And also a better use of the resources will be achieved, that leading probably to a reduction of the costs of the production.

This thesis makes a study of the biology and cultivation conditions of the rotifers, which is necessary previous to the application of control techniques, and then studies and proposes to different strategies for controlling the growth of the population, one based on the control of the feed density in the cultivation tank and the other based on the egg ratio control. In this work it is mainly done running simulations over a model, but an experiment is also performed for testing the second of the control strategies proposed.

Chapter 1

Introduction

Rotifers belong to the smallest metazoa of all the species that have been described. Their size rarely reaches 2 mm in length and males are smaller and less developed than females. In addition to their small size they posses a slow swimming velocity, which makes them a good prey for fish larvae. Besides, their tolerance to many different environmental conditions and high reproduction rate, among others, has made them to be a successful culture organism. Only a few rotifer species that belong to the genus *Brachionus* are used in aquaculture, among them the most widely used is *Brachionus plicatilis*.

1.1 Morphology

The epidermis of the rotifers contains a layer called the lorica, which shape and characteristics permit to determine the different species and morphotypes that exist. Three differentiated parts make up the rotifer's body: the head, trunk and foot. In the head the corona can be found. It has an annular ciliation and is retractable, which allows them to move and makes easier the intake of small food particles through a whirling water movement. The digestive tract, the excretory system and the genital organs are in the trunk. The foot is a ring-type retractable structure without segmentation and ends in one or four toes. In the figure 1.1 the body of a female and male from the *Brachionus plicatilis* strain is shown.

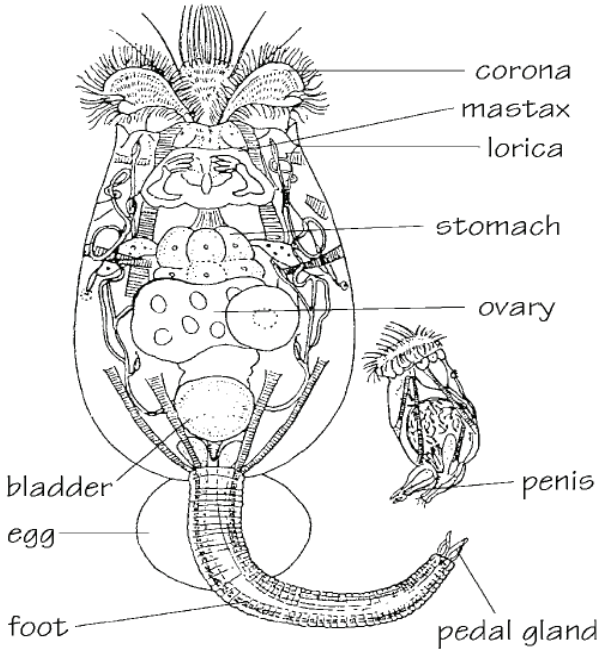

Figure 1.1: Brachionus plicatilis, female and male (from Dhert, 1996).

1.2 Life History

The life span of the rotifers, just as the reproduction activity, is dependent on their environmental conditions, for example at 25 ºC it is of about 4 days, while at a temperature of around 20–22 ºC the average life span is of 10.5 days. At 25 ºC the rotifers' larvae become adult after 0.5 to 1.5 days and then the females start putting eggs every four hours, approximately. After producing about ten generations females die.

The *Brachionus plicatilis* strain can reproduce both sexually (mictic) and asexually (amictic), depending on the conditions, environmental but also on the rotifer density of the population. During the mictic mode resting eggs are produced that will only develop and hatch, into amictic females, after they are exposed to specific conditions. This is probably a mechanism to preserve the survival of the population even under unfavourable conditions.

When talking about the growth rate of the rotifers, the mortality must be taken into account due to the short life-span of them. Thus, net growth rate is the difference between the gross growth rate and the mortality rate. The latter takes usual values between 0.1 day^{-1} for well-fed cultures and 0.16 day^{-1} for starved rotifers, for an average of 10.5 days of life (Y. Olsen, 2004).

1.3 Culture conditions

In this section the culture conditions of the rotifers will be considered, some of them related with the environmental conditions such as salinity or dissolved oxygen of the medium; and others related with the procedures, batch or continuous production for example.

1.3.1 Salinity

Brachionus plicatilis can live in mediums with a wide salinity range, from 1 to 97 ppt[1]. Though for reaching their optimal reproduction they must be in a medium with salinity below 35 ppt (Dhert, 1996). In case they must be fed to predators which need different salinity is better to acclimatize them before doing it, as a sudden change may cause a salinity shock and make them unable to swim, or even die.

Therefore, in this case a control of the salinity is not necessary, it would be enough to culture them with a known salinity water, the same of their predators if it is below the limit of 35 ppt to get an optimal reproduction. If that is not possible, use the closest salinity and acclimatize them before feeding the predators.

1.3.2 Dissolved oxygen

Rotifers can live in water with very low levels of dissolved oxygen, even in water containing just 2 $\frac{mg}{l}$ of dissolved oxygen (Dhert, 1996). Many factors affect to the level of dissolved oxygen, such as the salinity, rotifer density, temperature or type of food. Again, a control system for this variable does not seem to be implemented. It will be enough to set a constant aeration, which will also help to distribute the food all over the tank, but not so strong to damage, physically, the population.

1.3.3 pH and Ammonia (NH_3)

High levels of undissociated ammonia accumulated in the culture medium are toxic for the rotifers. This level is influenced by the pH and temperature of the culture water and concentrations below 1 $\frac{mg}{l}$ of NH_3 are safe (Dhert, 1996). Rotifers live in water with pH 6.6 or above, but the best results are obtained if the pH level is 7.5 or higher (Dhert, 1996).

The problem of the toxicity of ammonia has been solved by maintaining the culture pH at 7.0 with the automatic addition of HCl solution, thus contributing to the dissociation of NH_3 into $NH_4^+ + OH^-$ (Yoshimura et al., 1995), being this one less toxic to aquatic animals. For ultra-high-

1 ppt: parts per thousand (grams of salt per kg of water). This salinity unit is being replaced in many cases by psu (practical salinity units) which is based on conductivity measurements.

density production of rotifers ($1.6 \cdot 10^5$ $\frac{individuals}{ml}$) a system for continuous filtration, using a membrane filtration unit (pore size: 0,4 μm), for the elimination of ammonia has also been used (Yoshimura et al., 2003).

1.3.4 Bacteria and Ciliates

Bacteria and ciliates are not desired in the cultures. Though some kinds of bacteria can be beneficial to the rotifers (synthesizing vitamin B_{12}), they may not be to the predators and the transfer via the food chain can cause negative effects on them. If ciliates appear in the culture they will compete with the rotifers for the feed and, in addition, the metabolic wastes produced by the ciliates decrease the water pH level, which problems have been seen before.

However, the solution for these problems does not need any kind of control. In the case of bacteria a very effective way to decrease them consists on feeding the rotifers with *Lactobacillus plantarum* (Dhert 1996), while using phytoplankton filters (< 50 μm) or adding a low formalin concentration to the algal culture tank (if the rotifers are being feed with algae) are good solutions to the ciliates problem.

1.3.5 Temperature

The optimal temperature for culturing rotifers is dependent on the rotifer morphotype. For instance, *Brachionus plicatilis* (large or L-type) reach their optimal growth at lower temperatures, 18-25 ºC, than *Brachionus rotundiformis* (small or S-type), 28-35 ºC. Increasing the temperature within the range of optimal growth increases the reproductive activity, in general. On the other hand, increasing the temperature results in an increased cost for food, being necessary also to feed the rotifers more frequently in smaller distributions. This has to be done for keeping a good water quality, as well as to avoid periods of overfeeding or starvation, which at suboptimal temperature levels are not tolerated. On the other side, the use of temperatures below their optimal temperature slows down the population growth. In the table 1.1 the effect of temperature on the reproduction activity of the rotifers, *Brachionus plicatilis*, is showed.

Temperature (ºC)	15 ºC	20 ºC	25 ºC
Time for embryonic development (days)	1.3	1.0	0.6
Time for young female to spawn for the first time (days)	3.0	1.9	1.3
Interval between two spawnings (hours)	7.0	5.3	4.0
Length of life (days)	15	10	7
Number of eggs spawned by a female during her life	23	23	20

Table 1.1: Effect of temperature on the reproduction activity. (Dhert, 1996).

1.3.6 Cultivation feed and feed treatments

Brachionus plicatilis consumes most food particles of an appropriate size for consumption but, an efficient cultivation feed must also cover the nutritional demands of the rotifers and secure proper hygienic conditions in the cultivation tanks. Appropriate live feeds are microalgae, baker's yeast (alone or in combination with marine oils), and formulated diets that are commercially available.

Many species of microalgae are good food for rotifers, but their production costs are high. Typically, producers give microalgae as a component of the diet together with formulated diets or baker's yeast. That is because of the fact that small supplements of microalgae can contribute to better rotifer health and viability, reducing risks.

1.4 Culture procedures

The process of rotifer production is a combined production and n-3 HUFA enrichment, and involves several phases.

1. Maintenance of stock cultures.
2. Inoculation phase: start of new cultures based on inocula from stock cultures, or more commonly from production cultures.
3. Early growth phase: the critical phase when the food rations and rotifer density are increased gradually.
4. Late growth or production phase: the final phase when cultures are harvested.

1.4.1 Maintenance of stock cultures

Stock cultures should be kept physically isolated from the production facility of microalgae and rotifers in order to counteract contamination and the transfer of diseases. A sound precautionary approach that may reduce the risk of culture collapse and disease is to renew production cultures from the pure stock cultures at least once a year. Such a renewal should go along with complete hatchery disinfection.

Stock cultures of *Brachionus plicatilis* must not be contaminated by other zooplankton species. Most aquatic bacteria are harmless to rotifers and are acceptable in stock cultures, but well-known pathogenic bacteria should be excluded. Algal cultures used to feed the stock cultures of rotifers must also be free from harmful contaminants. Contaminated algal cultures are most easily rinsed by plating techniques using solid agar (Y. Olsen, 2004). Many species of microalgae used in aquaculture will grow on solid agar; other species are most easily purchased from culture collections that take professional care of the isolates (Y. Olsen, 2004).

Stock cultures of *Brachionus plicatilis* can be maintained in small units (0.1 – 1 litre). The water used must be sterilised. A stock culture is initiated by transferring 5 – 10 ml of mature stock culture to a beaker 0.1 – 0.5 l of sterilised water. The cultures can be maintained at room temperature, but the necessary feeding and renewal frequencies are lower if the rotifers are kept in the light at 7 – 10 ºC (strain dependent). The stock cultures will need to be renewed approximately once every month, or even less frequently at low temperatures.

If all the stock cultures become contaminated by other zooplankton, single rotifers should be selected under the microscope, carefully and repeatedly washed in sterilised water, and then transferred to small units containing sterilised water and microalgae. A thorough inspection is then needed to confirm success.

1.4.2 Inoculation phase

New cultures may be started using an inoculum taken from a production culture or from a stock culture. The quality of the production culture is important (e. g. egg · rotifer^{-1}, abundance of harmful micro-zooplankton, concentration of organic particles). A critical evaluation of the inoculum quality is an efficient precautionary measure against problems during later rotifer cultivation. One part of inoculum culture to 10 parts of water is suitable, and the culture (>30 rotifers · ml^{-1}) may be fed normal feed rations. The risk of severe rotifer mortality is highest during the first phase of the cultivation process. The risk may be reduced if the rotifers are fed with microalgae during the first one or two days after inoculation. Another way to reduce risks is to increase the initial inoculation density to >100 rotifers · ml^{-1}. A combination of high initial rotifer density, careful rinsing of the inoculum, and initial feeding by microalgae will normally ensure success.

The number of rotifers that is available is low if the inoculum is taken from stock cultures. A suitable method is then to grow the rotifers with microalgae only during the first few days (densities >2 rotifers · ml^{-1}). This is easily done by inoculating them with microalgae in illuminated rotifer tanks (light tubes or other sources, >100 W · m^{-3}) at 10% strength of a normal algal medium (formulations in Smith *et al*., 1993; Coutteau, 1996). The main production feed should be added before the algae become grazed down by the rotifers, but the rotifers should be fed algae for circa two days. This method is also appropriate if the quality and viability of the production cultures are

poor. The green algae *Tetraselmis* spp. have been shown to support culture self-cleaning quite efficiently. Many contaminating micro-zooplankton species cannot ingest the large *Tetraselmis* cells very efficiently, and will probably be out-competed by rotifers after the change in food source.

1.4.3 Early growth phase

The first 2 – 6 days of the cultivation process, when food rations and rotifer densities are raised, has been shown to be the most critical phase of rotifer production. As mentioned above, prophylactic measures to counteract potential problems are high initial rotifer densities and thorough quality evaluation of the inoculum. A further serious problem of the initial phase is related to a mismatch between the food ration offered and the rotifers' food requirements of growth. Feeding a high specific food ration (food per rotifer per day) is important in order to obtain a rapid growth rate and viable rotifers. On the other hand, overfeeding may cause unfavourable environmental conditions (low oxygen, high reactive ammonia and extensive bacterial growth) and enhanced rotifer mortality.

Feeding during the early phase of growth must be based on the rotifers' actual food requirements for growth. The specific growth rate of rotifer cultures for a given feed is a function of the specific food ration (µg yeast · rotifer^{-1} · day^{-1}) supplied. The rotifer cultures must be fed approximately 0.5 µg baker's yeast (plus 0.05 µg oil) per rotifer per day, or 0.5 day^{-1} (food concentration per rotifer concentration and day) in order to maintain a positive net growth rate (Y. Olsen, 2004). The growth response is comparable for batch and continuous cultures when the feeding rate is below 1 µg per rotifer per day, yielding growth rates below 0.2 day^{-1}. For higher food rations, the growth response for a given food ration becomes dependent of the cultivation method used. The lower specific growth rates obtained in continuous cultures are primarily the result of higher feed losses compared with those of closed batch cultures. Cultures harvested continuously at high rate are characterised by high water turbidity, which means high losses during water exchanges. The batch cultures are closed and the food is better utilised under high feeding conditions.

1.4.4 Late growth phase

The methods used to produce rotifers after the early phase of increasing rotifer densities are highly diverse. From a general point of view, two distinctively different methods can be defined, and claim that most methods used are combinations of these principal cultivation methods.

1.4.4.1 Batch cultures

Consists on the production in a closed culture system supplied only with the resources needed for growth. Cultures are completely harvested at a certain developmental stage or time in the late phase of growth.

Growth in batch culture during the early and late phases involves variable and transient

conditions for the rotifers. Initially are exposed to sufficient food and a promising prediction of life. Later, when the cultures are fed the maximum sustainable feed ration, they experience severe food limitations, reduced growth rates and enhanced mortality. In the transition phase, they undergo major changes in their individual biomass, biochemical composition and nutritional value.

The rotifer density will stabilise at a constant level when a constant amount of feed is added daily to the rotifer culture for a period of time. This level denotes the carrying capacity at the given feeding conditions. The maximum feed ration used during the late phase of growth affects the stationary biomass level but not the initial specific growth rate of the cultures, which is determine by the feeding conditions during the early phase. The carrying capacity increases linearly with increasing food ration. Some care should be taken, but increased sedimentation of feed and enhanced density of dead particles in the cultures will most probably accompany deviations from the general linear relationship.

Rotifer production in batch cultures calls for a harvesting strategy that secures both efficient production and acceptable live-food quality. The increase in rotifer density is exponential in the initial phase, and then levels off at a stationary value. From a production efficiency perspective, harvesting should not be undertaken before the culture has reached its optimum for net production. At that time, the rotifer density will be 50% of the carrying capacity, and the net growth will be half the maximum specific growth rate. The net production rate is also acceptable during the first days following the production optimum. The nutritional value is rapidly reduced after 5 – 7 days. In practical rotifer production, a time window for harvesting is more feasible than one single day. An overall evaluation suggests that batch cultures should be harvested when the rotifer density is between 50% and 75% of carrying capacity of the culture (Y. Olsen, 2004).

1.4.4.2 Continuous cultures

Consists on the production in an open culture system that is supplied with the necessary resources and harvested regularly by replacing a fixed volume of the culture by seawater once daily, or continuously, during the late phase of growth.

Both methods and appropriate combinations are feasible for production. The priorities of the producer regarding rotifer quality, costs, risks and hatchery/laboratory routines may affect the choice of method. Adequate knowledge of rotifer biology, their nutritional and environmental requirements, and the traits of the specific cultivation system are important for sustainable and safe mass cultivation of rotifers.

Chapter 2

Material and Methods

2.1 Instrumentation

For the experiments, different kind of information is needed. Some of the data is used by the controllers and other is important to know the state of the population, as was explained in the culture conditions section. In the following points the different instruments used and its characteristics are explained.

2.1.1 Turbidity sensor

During the experiments, measurements of the feed density had to be performed. Measuring the turbidity of the water was thought to be a good way of having measurements of the feed density in the cultivation tank. And for this purpose two turbidity sensors have been used, both from the company *Mettler Toledo®*, specifically the models from the *InPro®8000* series: *InPro®8050* and *InPro®8200*. Both of them make use of the transmitter *Trb8300*.

Figure 2.1: InPro®8200 sensor.

The sensor of the *InPro®8000* series are based on the principle of backward scattered light. The *Trb8300* transmitter is equipped with a LED which beams a light which is almost in the infra-red range (wavelength 880 nm) via a fibre optic cable into the liquid medium. If the light impacts on particles, the light is scattered in all directions. The light scattered at and angle of 180º is captured and led back via a fibre optic cable to a photodiode in the transmitter, processed and the signal transformed into a value for the display. The *InPro®8050* sensor is a single optical fibre sensor, while the *InPro®8200* sensor is a dual optical fibre sensor, which means that the former uses the same fibre as transmitter and receiver and the latter uses one of the fibres for transmitting and the other for receiving.

The table 2.1 shows a resume of the technical data of both sensors.

	InPro®8200	***InPro®8050***
Measurement principle	Dual fibre	Single fibre
Window material	Sapphire	Sapphire spigot
Measuring range	5 FTU ... 4000 FTU 0 ... 30 g/l	10 FTU ... 4000 FTU 0 ... 250 g/l
Pressure range	0 ... 6 bar or 0 ... 12 bar (with Epoxy bonded window)	0 ... 2 bar
Temperature range	-10 ... 130 ºC or -30 ... 130 ºC (with Epoxy bonded window)	0 ... 60 ºC

Table 2.1: Resume of the technical data of the turbidity sensors.

Regarding to the transmitter, some of its main features are the following:

- Three retrievable, independently configurable parameter sets.
- Manual, process and multipoint calibration procedures.
- Four 0/4 ... 20 mA galvanically isolated outputs.
- RS232 interface for software updates and printing of configurations.

Figure 2.2: Trb3000 transmitter.

And its technical specifications are shown in the table 2.2.

Trb3000	
Power supply	100 ... 240 VAC (25W max)
Light source	LED, emitting frequency 880 nm
Measurement	Input/output for InPro8000 series, backscattered light principle
Measurement range	5 FTU ... 4000 FTU 0 ... 250 g/l
Selectable measuring units	FTU NTU EBC g/l % ppm

Table 2.2: Technical specifications of the transmitter Trb8300.

2.1.2 Thermometer

Figure 2.3: Fluke® 51 II Thermometer.

For measuring the temperature of the water inside the cultivation tank the *Fluke® 51 II Thermometer* with a K type thermocouple (model *80PK-1*) is used. It has an accuracy of $\pm[0.05\% + 0.03\,^{o}C]$. The thermocouple is a K type, Chromel Alumel, bead style with a range of measurement from -40 ºC to 260 ºC and an accuracy of $\pm 1.1^{o}C$.

2.1.3 Oxygen

The oxygen is an important value to measure as the density of the population increases and more food is added to the tank. For measuring the amount of dissolved oxygen the *OxyGuard® Handy Polaris* is used.

The sensor gives the measurements in $\frac{mg}{l}$ and % saturation and has a measuring range of $[0-60.0]\frac{mg}{l}$ and a working range of temperatures between -5 ºC and 45 ºC with an accuracy $\leqslant 1\,\%$ of the measured value.

The probe is a galvanic type probe that does not need warm up time and is temperature compensated.

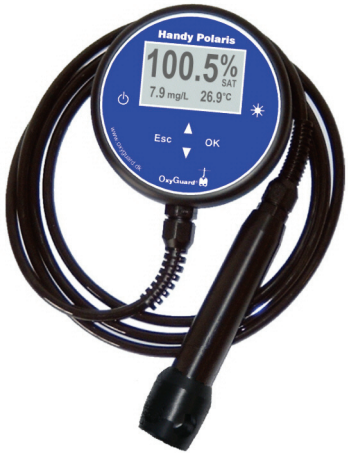

Figure 2.4: OxyGuard® Handy Polaris.

2.1.4 pH

The measures of pH are done using *Merck® Neutralit®* stripes, that have a measurement range between pH 5 and pH 10 with a 0.5 interval.

2.1.5 Salinity

For measuring the salinity the *ATAGO® S/Mill* salinity refractometer is used, which measures within a range from 0 to 100 ‰ with an accuracy of ± 1 ‰ and is temperature compensated.

2.2 Rotifer model

For all the simulations done during this project a model developed by Morten Omholt Alver has been used. This is an individual-based model which takes into account the body composition of the rotifers, representing the nutrients in separate energy reserve compartments and defining stoichiometric rules for growth based on the balance between these.

The model has three reserve compartments, E_P, E_L and E_C:

- E_P: energy reserves, protein compartment
- E_L: energy reserves, lipid compartment
- E_C: energy reserves, carbohydrate compartment

and seven state equation in total: V, R, E_P, E_L, E_C, M_Q and h, being V the structural volume, R the reproductive buffer, M_Q aging and h the hazard rate.

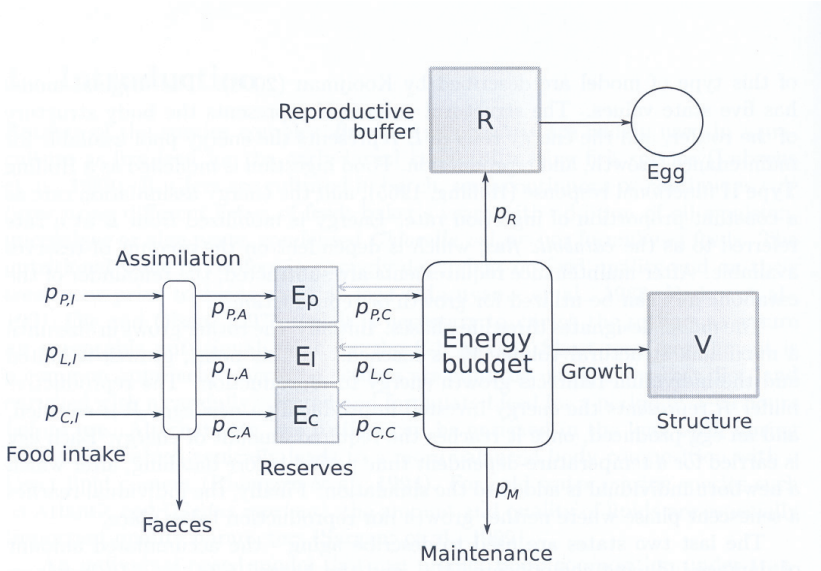

Figure 2.5: Overview of the individual model (from Alver, M. O. Doctoral thesis, NTNU).

The model assumes that maximum feed intake is given as a surface area-specific energy flux, what implies that maximum feed intake by weight will decrease with increasing energy density of the feed, and that maximum gross energy ingestion is independent of feed composition. Feed ingestion is modelled as a Holling type II functional response. Feed composition is defined by protein, lipid and carbohydrate content relative to dry weight, each of them having a specific ingestion rate ($p_{P,I}$, $p_{L,I}$, $p_{C,I}$) and assimilation flux ($p_{P,A}$, $p_{L,A}$, $p_{C,A}$).

The catabolic fluxes ($p_{P,C}$, $p_{L,C}$, $p_{C,C}$) represent the rate of expenditure of the energy reserves. The catabolic fluxes and the growth rate $\frac{dV}{dt}$ are interdependent.

The maintenance requirement is proportional to V and is managed by a synthesizing unit that can operate on protein, lipid or carbohydrate, but with different preference for each. The maintenance synthesizing unit produces a flux equal to P_M by using the available fluxes of protein, lipid and carbohydrate ($p_{P,C}$, $p_{L,C}$ and $p_{C,C}$).

After subtracting maintenance fluxes, the rest of the catabolic fluxes are available for growth or reproduction. The stoichiometric requirements for growth are set according to the dry weight fraction of protein, lipid and carbohydrate in structure. The remaining part of the fluxes after subtracting the fluxes used for growth are in part be excreted, and in part returned to their energy reserves compartments.

In the reproductive phase, $\frac{dV}{dt}=0$, egg production follows the same principles as growth, except that the result is accumulated into the reproductive buffer *R*. When the buffer contains an amount of energy equal to that required to produce an egg, it is emptied and an egg is immediately produced.

Aging is related to cell damage-inducing components accumulated through catabolism. The amount of these components is M_Q, and their rate of accumulation is proportional to the catabolic rate. The hazard rate *h* represents accumulated cell damage, and increases as a function of the concentration of damage-inducing components.

For taking into account the dependence of the temperature, the rate parameters are multiplied by a temperature correction factor T_C:

$$T_C=\frac{e^{\left(\frac{T_A}{T_1}-\frac{T_A}{T_w}\right)}}{1+e^{\left(\frac{T_{AL}}{T_w}-\frac{T_{AL}}{T_L}\right)}+e^{\left(\frac{T_{AH}}{T_H}-\frac{T_{AH}}{T_w}\right)}}$$

where T_w is the water temperature, T_1 is the reference temperature, and T_L and T_H relate to the lower and upper boundaries of the tolerance range. T_A, T_{AL} and T_{AH} are Arrhenius temperatures.

The feed concentration model accounts only for additions, dilutions and ingestion by rotifers, thus:

$$\frac{dX}{dt}=addition-\frac{ingestion}{tank\ volume}-dilution$$

where the ingestion by rotifers is calculated and added together for all individuals.

2.3 Growth control

The objective is to control the growth of the rotifer population, so its dynamics can be controlled and make it predictable, as well as improve the conditions at which rotifers are cultivated.

In this thesis it has been studied from two points of view, one in which an automated method of knowing the feed density in the tank is available and other without this kind of instrumentation available.

2.3.1 Feed controller

The first of the studied control strategies for the application to the rotifer cultivation is based on the control of the food added to the tank in an automated way, what can help to avoid periods during which rotifers are reared with an excess of food in the tank and periods of starvation; allowing to have them in good and as stable as possible conditions during all the process; and use the most accurate amount of food possible to feed the rotifers.

The proposed control strategy is made up of two control loops. An inner loop which will make the tank to contain as much feed as set by a set point and; an outer loop that will vary that set point depending on the actual growth rate.

The inner loop consists of a Proportional-Integral-Derivative (PID) controller and a Feedforward term so that the controller can react to the error more quickly in order to bring the system to the desired reference. A Gain Scheduling is also used to modify both terms, the PID and the feed forward, as long as the behaviour of the system is dependent on the rotifer density. The purpose of this loop is to make the feed density in the tank be the same as the reference, and it is not intended to affect directly to the dynamics of the system, this is the task of the outer loop. In the following figure a block diagram of this loop is shown.

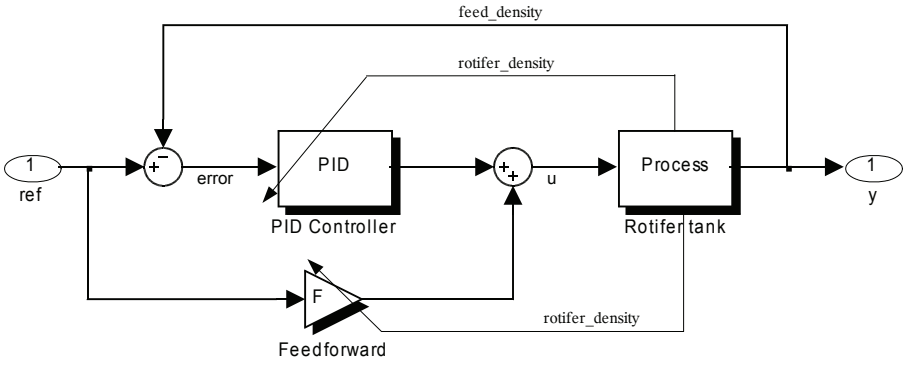

Figure 2.6: Control loop for the feed density.

The general equation for a digital PID controller is the following:

$$u_{PID} = (K_p \cdot e) + (K_i \cdot \Sigma e) + (K_d \cdot (e - e_{k-1}))$$

being e the error and K_p, K_i and K_d the proportional, integral and derivative constants respectively. In the case of this controller the K_p, K_i and K_d are not constants but parameters whose values depend on the rotifer density inside the cultivation tank.

The same happens with the feedforward term, which will have a variable value depending on the rotifer density. Thus, the equations of these terms can be written as:

$$u_{PID} = (\bar{K}_p \cdot e) + (\bar{K}_i \cdot \Sigma e) + (\bar{K}_d \cdot (e - e_{k-1}))$$

$$u_F = \bar{F} \cdot ref$$

$$u = u_F + u_{PID} = (\bar{F} \cdot ref) + (\bar{K}_p \cdot e) + (\bar{K}_i \cdot \Sigma e) + (\bar{K}_d \cdot (e - e_{k-1}))$$

and the values for the parameters after the tuning of the controller are:

$$\bar{F} = \begin{cases} 0.000625 & \text{if rotifer density } < 100 \ \ rot/ml \\ 0.001 & \text{if rotifer density } > 100 \text{ and } < 225 \ \ rot/ml \\ 0.0015 & \text{if rotifer density } > 225 \ \ rot/ml \end{cases}$$

$$\bar{K}_p = \begin{cases} 1.75 & \text{if rotifer density } < 225 \ \ rot/ml \\ 2.0 & \text{if rotifer density } > 225 \text{ and } < 300 \ \ rot/ml \\ 2.25 & \text{if rotifer density } > 300 \ \ rot/ml \end{cases}$$

$$\bar{K}_i = \begin{cases} 0.18 & \text{if rotifer density } > 225 \text{ and } < 300 \ \ rot/ml \\ 0.15 & \text{in any other case} \end{cases}$$

$$\bar{K}_d = 0.06$$

As can be seen in the figure 2.6, in order to run this controller is necessary a measurement of the feed density inside the tank, as well as the rotifer density. During the simulations it is assumed that such sensors are available and the data from the model will be used as the output from them.

The outer loop works in the following way: it runs every twenty-four hours and takes the value of the rotifer density and depending on this value sets the growth rate reference. This value is compared with the actual growth rate of the population, which is calculated using the egg rate value (eggs per rotifer) of the population as follows:

$$\mu_{net} = 0.90 \cdot ER - 0.16$$

(Y. Olsen, 2004), where μ_{net} represents the net growth rate, ER the egg rate and 0.16 (day^{-1}) is a estimated mortality rate value. With these values, the error between the growth rate reference and the real growth rate is calculated and, in this case, a proportional controller is used to modify the feed reference. In the next figures the outer loop controller and the whole growth rate controller block diagrams are shown.

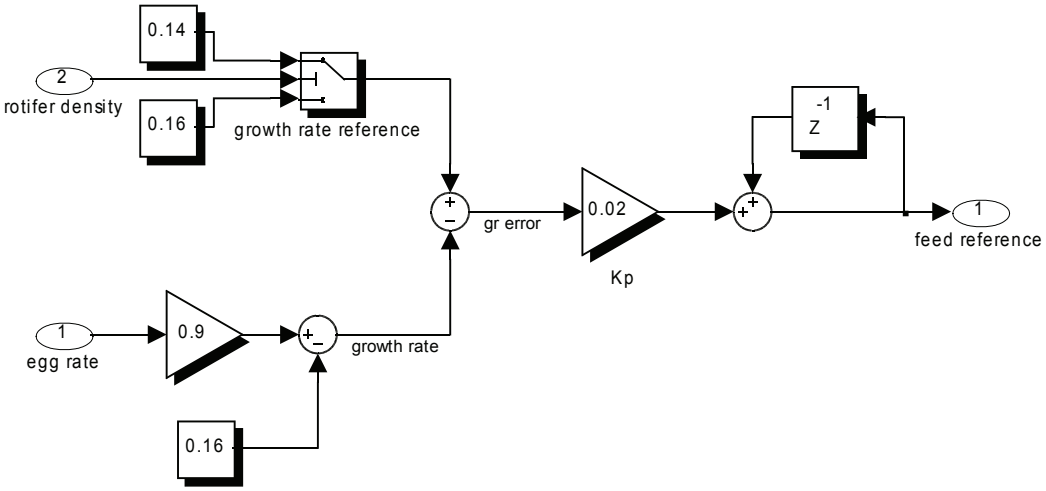

Figure 2.7: Feed reference controller.

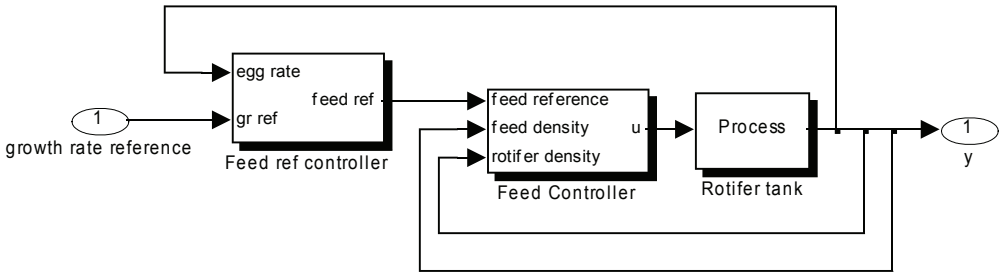

Figure 2.8: Growth rate controller by means of Feed density control.

Thus, the equations of the control algorithm of the external loop are:

$$gr = 0.90 \cdot ER - 0.16$$

$$gr_{error} = gr_{ref} - gr = gr_{ref} - (0.90 \cdot ER - 0.16)$$

$$feed_{ref} = feed_{ref(k-1)} + (0.01 \cdot gr_{error})$$

Where the growth rate reference is a parameter which value depends on the rotifer density measured. The way it varies can be modified according to the desired experiment: try to keep the population in a determined rotifer density range, get the maximum growth of the population, use the

minimum amount of food , etc. So, depending on the desired results the law modifying the growth reference set point it will have to be studied. For our purpose, as a first study of this kind of control for the rotifer cultivation, of achieving a rotifer density in a determined range a switching law is used.

One of the objectives was to implement this controller and run an experiment but, as it will be explained, after many tries it was not possible to get measurements of the feed density and, therefor, only simulations could be performed of this controller.

2.3.2 Egg Rate controller

The second control strategy studied is based in the egg rate control without the knowledge of the feed density in the tank, making use only of the rotifer density and egg rate of the population. In this controller the controlled parameter is the egg ratio, instead of the growth rate but, as it has been shown before, there is a direct relationship between both values so, controlling the egg ratio the growth rate is being controlled but, as the conversion to growth rate is not exact and the measured value is the egg rate, that is the one used in the controller. In the figure 2.9 an overview of the controller can be seen.

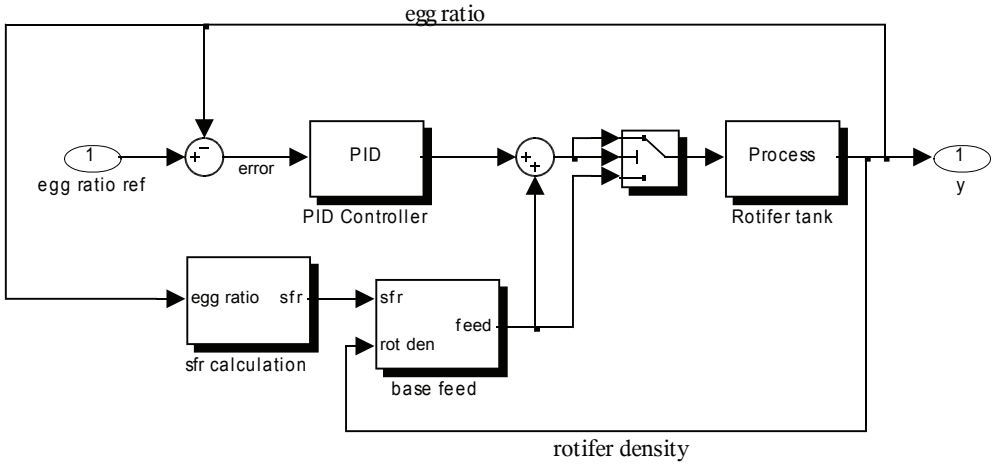

Figure 2.9: Overview of the egg rate controller.

Basically it is a PID controller that modifies a base feed calculated on the basis of the egg ratio and the rotifer density. The calculation of the base feed needs two data, the specific food ration (SFR, food per rotifer per day) and the total number of rotifers in the tank. The former is obtained from the net growth rate that, as seen above, can be obtained from the egg ratio; and the latter is calculated with the rotifer density and the volume of water used. Finally, to get the base feed a constant is needed as the base time unit used in the model is one day and the feed is going to be added every half an hour. That is:

$$SFR = \frac{0.184}{(0.377 - \mu_{net})}$$

$$\mu_{net} = 0.9 \cdot ER - 0.16$$

$$SFR = \frac{0.184}{(0.377 - \mu_{net})} = \frac{0.184}{(0.377 - (0.9 \cdot ER - 0.16))}$$

$$SFR = \frac{0.184}{(0.537 - 0.9 \cdot ER)}$$

$$rot = rot\_den \cdot V \cdot 1000$$

$$feed = SFR \cdot rot \cdot K$$

The SFR has a maximum value of 1.6 µg as long as it is directly related with the egg ratio, to avoid an excessive increase of the value, and also because when the product 0.9·ER gets values close to 0.537 the SFR value will tend to infinite. And also a minimum value that would depend on the reference, which will be used in case of obtaining a negative SFR (that will happen when 0.9·ER is higher than 0.537).

After the base feed is calculated, its value is modified by the PID controller, which parameters have been modified after the experiment performed, being finally:

- $K_p = 0.006$
- $K_i = 0.03$
- $K_d = 0.12$
- Saturation of the integral action = ref / 6
- Saturation of the derivative action = 0.0144

And finally a selection is made between the modified value or the base feed in case that the modified value is negative, if this is the case, the base feed is used; in other case the value modified with the PID controller is used.

After the simulations were done, an experiment using a 250 litre tank and of 12 days length was run. The rotifer density and egg ratio were measured manually once per day. To reduce the error in these measurements, three measurements from three different samples were done and then, the average value was used as the value measured. For measuring, from a sample, 12 drops of 50 µL each one, taken with a mechanical pipette with adjustable volume, are taken and with a microscope, the number of rotifers and eggs in each one are counted. Then, the higher and lower values are discarded and the resting ten values are added, having the number of rotifers in half millilitre, that value is multiplied by two, obtaining this way the rotifers per millilitre; the number of eggs counted is divided into the number of rotifers counted and the egg ratio is obtained. Initially, the idea was using a rotifer counter (Alver et al., 2006) for having automatic and more precise measurements, but it was not possible to make it run and, therefor, the count had to be done manually.

Due to the lack of time it was not possible either to get the material to use the computer for controlling the feed, thus, the feed was added to the tank continuously, instead of 30 minutes intervals as in the simulations, using a pump and configuring the speed to make the reservoir of food 24 last hours, so the same amount of food during a day would be fed, but continuously. For the feeding, the amount of yeast was calculated as in the simulations, but a 10% of rotifer diet (based on algae) was added to a better rotifer health and viability, reducing risks.

Daily measures of the temperature, dissolved oxygen, pH and salinity were also done to have information about the state of the population. And a daily 30 % dilution starting the sixth day was done.

Chapter 3

Results

3.1 Temperature simulations

Using the rotifer model built by Morten Omholt Alver simulations of the effect of different temperatures in a rotifer culture has been done. Rearing temperatures of 22 ºC, 25 ºC, 28 ºC and 31 ºC have been used with the *Brachionus plicatilis* strain during 25 days. The two first ones are within the range of optimal temperatures for rearing this strain, being 25 ºC in the limit of it, while 28 ºC and 31 ºC are a bit above the range. In all four cases the initial conditions where the same, starting with a concentration of 100 individuals per millilitre from the same previously existing population in a 100 litre of water tank. A 13.0 $\frac{mg}{l}$ reference for the feed concentration during all the experiment has been used for the four temperatures, using the feed controller explained previously to achieve this. During the simulations a 20 % of the water is diluted (with the result of a decrease of the population as well) from the sixth day of the experiment. In the following images, the results of these simulations can be seen.

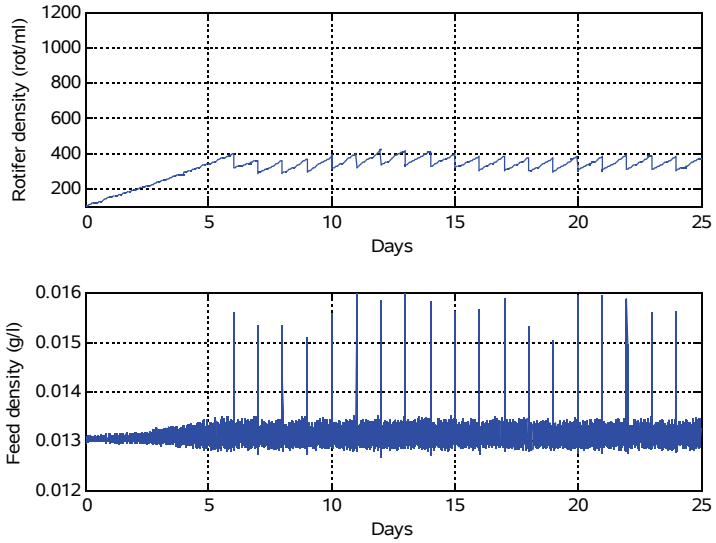

Figure 3.1: Simulation results at 22 ºC.

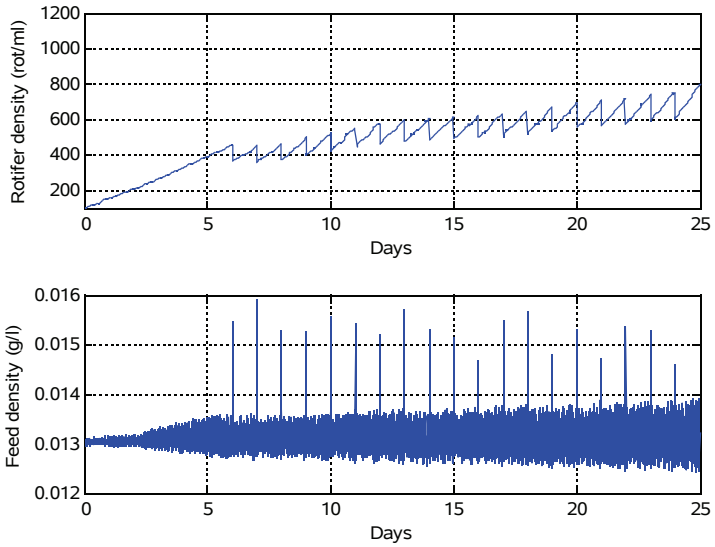

Figure 3.2: Simulation results at 25 ºC.

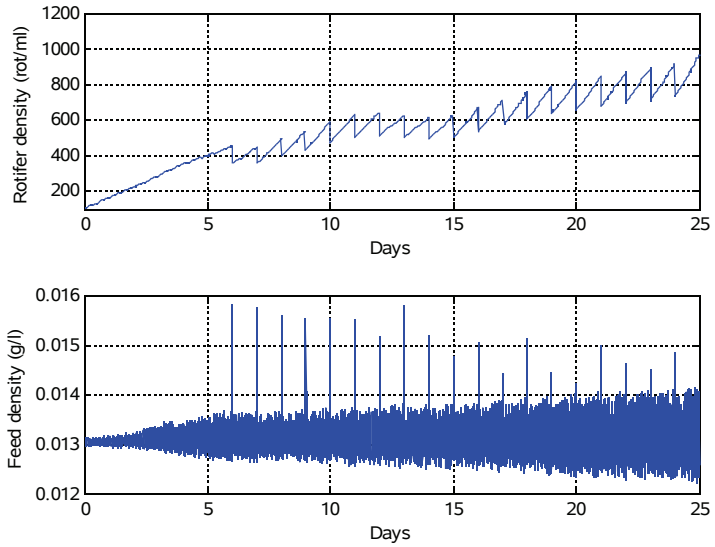

Figure 3.3: Simulation results at 28 ºC.

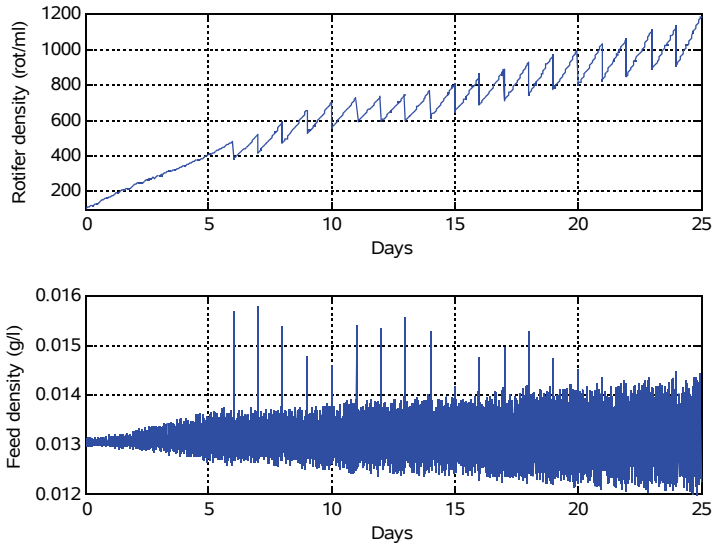

Figure 3.4: Simulation results at 31 ºC.

As can be seen at first sight, as the temperature increases the same happens to the growth of the population and the food intake. In a more detailed view of the data obtained from the simulations can be seen that the final concentration of rotifers after 25 days at 22 ºC is 370 individuals per millilitre, and an amount of 8.7362 grams per litre were added (870.62 grams in total) at the end of the experiment; at 25 ºC the population reached a value of 795 rotifers per millilitre, with 14.9308 grams per litre of yeast added (1493.08 grams in total); 967 rotifers per millilitre was the value at 28 ºC, with the addition of 17.9790 grams per litre (1797.90 grams in total) of yeast; and finally at 31 ºC, 1179 rotifers per millilitre were reached and 22.3535 grams per litre (2235.35 grams) of yeast were added.

A very noticeable increase of the population happens when using 25 ºC instead of 22 ºC: more than the double (a 114.8 % increase) of the final concentration. Also a great increase of the feed happens, a 70.90 % more of yeast is needed, but the increase of the population is much higher than the increase of the food used.

On the other hand when using a rearing temperature of 28 ºC, the population obtained is a 21.635 % higher than at 25 ºC, while the use of food is a 20.416 % higher, while using a temperature of 31 ºC the final rotifer density is a 21.923 % higher than at 28 ºC and the food used is a 24.331 % higher. So, a temperature above the optimal range can give a higher growth rate, but the cost of food has the tendency of increasing faster the growth of the population. In the case of rearing during long time and with constant temperature it does not seem to be appropriate to use temperatures that are not within the optimal range for the strain used.

A last simulation was done to see the effect of the temperature when using a temperature further beyond the range of optimal temperatures, in this case a 40 ºC temperature was used, and the results are showed in the figure 3.5. The final population reached the 913 rotifers per millilitre, what is lower than the density reached using 28 ºC, and the use of yeast was 17.3163 grams per litre That confirms what has been said about using temperatures out of the optimal range, specially as we go further and, besides, if it is taken into account that the use of energy for reaching higher temperatures.

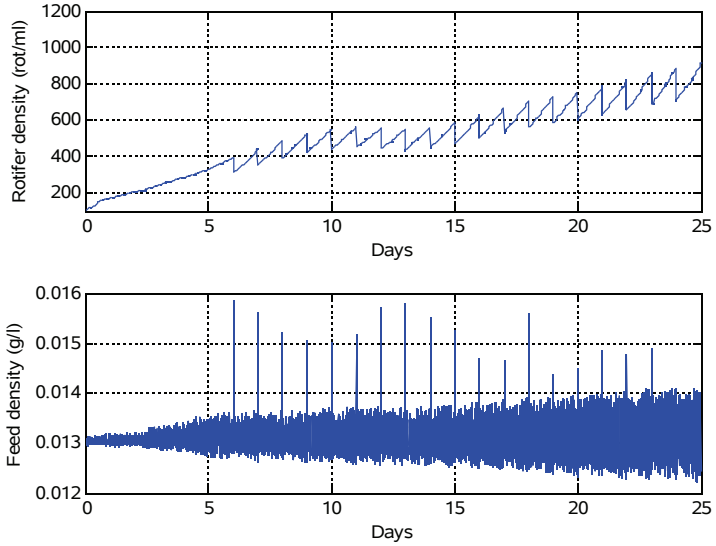

Figure 3.5: Simulation results at 40 ºC.

Thus, the temperature at which rotifers are reared has a direct influence in the population dynamics, making it a parameter which could be of interest from a control point of view, and not only for using a constant temperature during a whole experiment, as in the previous. For example, during the first days of the production a higher growth rate could be interesting, so the rotifer population would increase quickly and reach high concentrations in just a few time; then when a desired value is reached, slow down their growth to keep them around that value. That could be done controlling the temperature of the culture water, using a high value in the first stage and decreasing the temperature later.

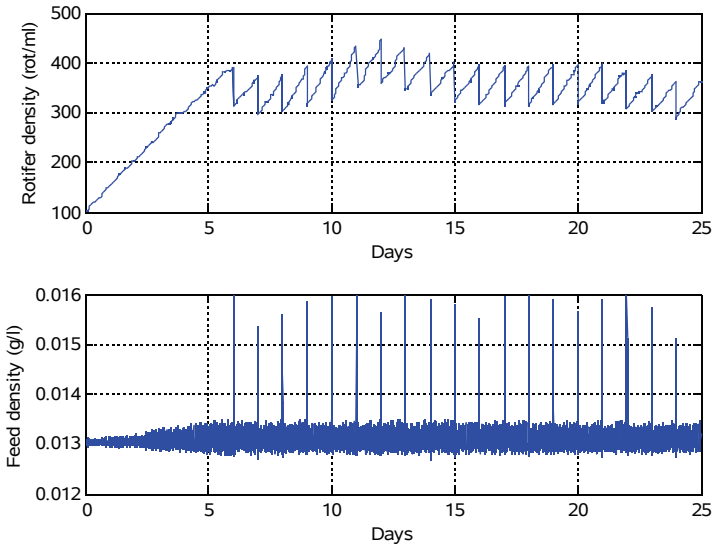

Figure 3.6: Starting temp. of 25 ºC, switching to 22 ºC when population reaches the 300 ind. · ml^{-1}.

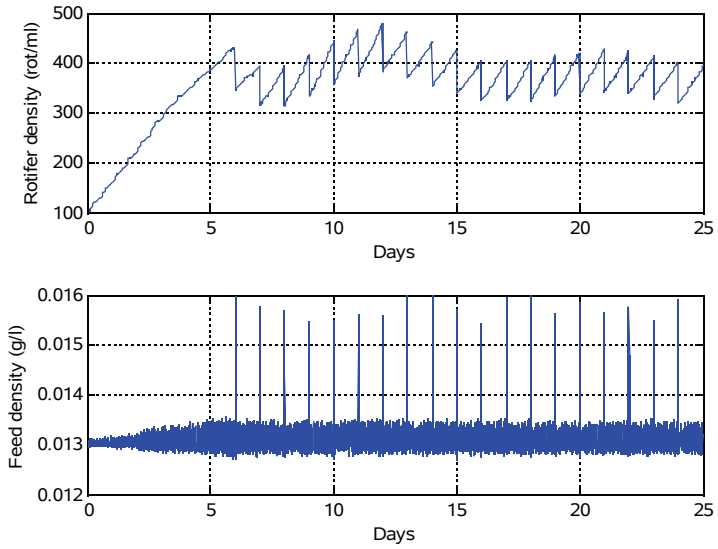

Figure 3.7: Starting temp. of 28 ºC, switching to 22 ºC when population reaches the 300 ind. · ml^{-1}.

In the figure 3.6 can be seen the results of using a temperature of 25 ºC until the population reaches 300 individuals per millilitre, then the temperature of the culture water is reduced until 22 ºC to keep it in that level.

As expected, a combination of both dynamics is obtained, with a high growth rate while the rotifers are reared at 25 ºC and then, a reduced activity, keeping the rotifer density level in the range between 300 and 400 individuals per millilitre, when the temperature at which are reared is 22 ºC. At the end of this simulation the amount of yeast used is 9.1549 grams per litre (915.49 grams in total).

The use of temperatures above the optimal range did not seem a good choice when using them during a whole rotifer cultivation but, looking at the above figures, it seems more justified when a high growth rate is desired at low densities only, like in the last experiment. In figure 3.7 an experiment like the last one is showed but, in this case the starting temperature is 28 ºC instead of 25 ºC. As before, when the concentration of rotifers achieves 300 individuals per millilitre the temperature is changed to 22 ºC.

The amount of yeast used in this case is 9.8501 grams per litre (985.01 grams in total), just a bit higher than previously but, due to the higher activity of the rotifer population, the level of 300 rotifers per millilitre is obtained at the third day, while this level at 25 ºC is reached one day later. After that, the density during the rest of the experiment continues being somewhat higher than when 25 ºC were used as the starting temperature, with the final use of just 70 grams more of food in 25 days.

3.2 Turbidity

Experiments with both sensors were done for trying to measure the turbidity within the range of feed concentrations that were supposed to be used during the experiments with a real rotifer population, that means that the maximum value wanted to be measured was about 30 mg/l of food, and an accuracy of at least 0.1 mg/l would be desirable.

Offline calibrations, using small 1 litre and 10 litre receptacles, as well as online calibrations, installing the sensors in the tank that was intended to be used with the rotifers' experiments, were performed. During these calibrations all three methods available were used: manual, where offset and slope must be set manually; process, where the zero point is set manually and the slope is set by indicating the value of the turbidity of the sample being measured; and multipoint, in which 2, 3, 4 or 5 samples are used and the value of the turbidity of this samples has to be introduced. Also different ranges for the calibration were used, using as the top value samples up to 1 g/l, and as measuring units, mainly g/l, but also % in some of the experiments.

With the *InPro®8050* sensor it was not possible to get measurements below 150 – 200 mg/l, with the readings dropping to 0 under these values. That values are far from the needs for the experiments with a real population.

Regarding to the *InPro®8200* sensor, it was possible to get readings up to 80 – 100 mg/l using only yeast and, using algae instead of yeast, the measurements improved being possible to get values up to densities around the 50 mg/l and then, the readings dropping to 0 again. Even if it was an improvement, it was not god enough for the requirements we had.

3.3 Feed controller

As is has been explained previously, due to the fact that the feed density was not possible to use, only simulations could be performed, with the following results. First of all, simulations with different feed density set points were performed for both adjust the parameters of the controller and see the differences in the population dynamics. In the figures 3.8, 3.9 and 3.10 are shown the results of using 0.010, 0.013 and 0.024 grams per litre All of them were performed with the same conditions as the ones in the temperature section, except that in these ones, the temperature was constant at 25 ºC during all the time. In these simulations only the inner loop of the controller is used, as long as the reference is not modified at all. In the first of them, when the 20 % dilution starts the sixth day, the population growth cannot compensate it and the density is reduced every day, obtaining a final value of just 196 individuals per millilitre In the other two cases the population keeps growing after the sixth day, slowly in the case of the 0.013 grams per litre reference and very fast in the last case. That behaviour is due to the fact that at a specific feed density, the population tends to achieve a determined egg ratio which, as seen before, is directly related with the growth rate. In the first of these simulations it is obvious that the growth rate is lower than the one required to, at least, produce as much rotifers as are being diluted. And it also makes clear that for a given dilution rate there will be always a specific feed density that will produce approximately the same amount of rotifers being diluted and keep the rotifer density almost constant.

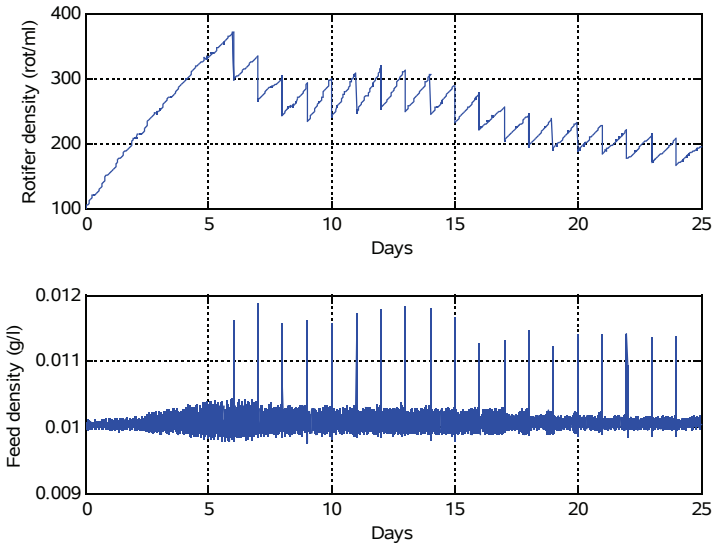

Figure 3.8: Simulation results with 0.010 g/l reference.

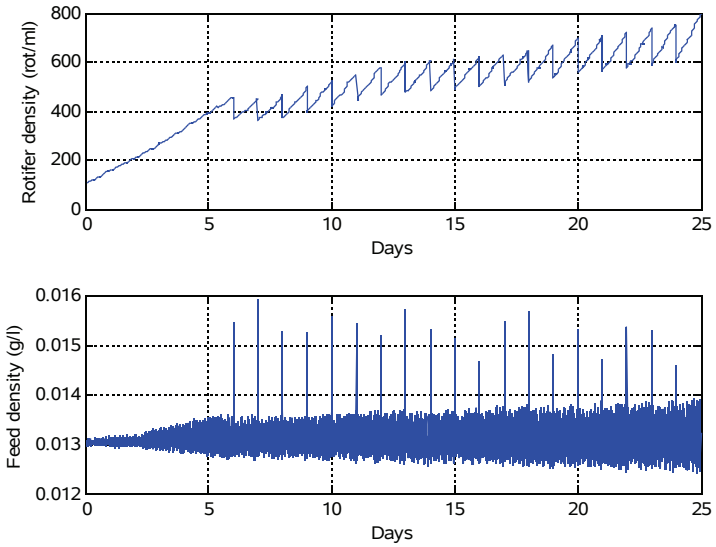

Figure 3.9: Simulation results with 0.013 g/l reference.

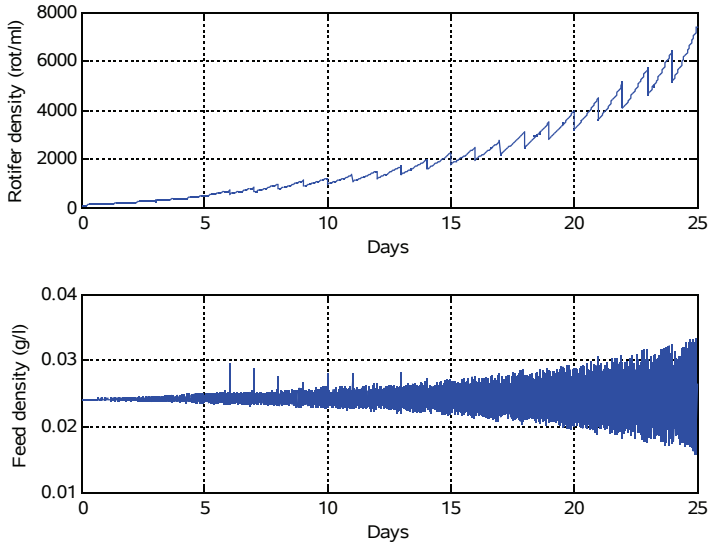

Figure 3.10: Simulation results with 0.024 g/l reference.

In this last one picture is obvious that the feed controller cannot keep the feed reference as well as in the other cases, but it is just because of the controller is not intended to work with such densities and, thus, it was no prepared for them. Anyway, it is not important for the results of these simulations. In figure 3.11 the egg rate of all three simulations is shown, clearly showing the results seen above.

For the 0.010 g/l reference, the steady egg ratio is about a value of 0.3 egg/rot. According with the previously introduced formula relating egg ratio and net growth rate of about 0.11, or 0.17 if the mortality value is supposed to be of 0.1 instead of 0.16, not being enough for compensating a loss of a 20 % of the population every day.

In the second case, the one with a 0.013 g/l reference, the egg ratio after the first period of fluctuation is about a 0.4 egg/rot. Calculating with the formula the result is a net growth of 0.2, what would make the population not to grow after the dilution starts, thus being clear that the mortality is lower than the 0.16 used in the formula and having a real growth rate somewhat higher than the 0.2, and also explaining why the growth of the rotifer population is slow but quite constant compared for example with the last of the simulations.

At last, when using a 0.024 g/l reference the egg ratio is a bit higher than 0.5 egg/rot, giving a growth rate value of about 0.29 or, surely in this case, higher, having a daily net increase of the population and having that characteristic exponential-form curve for the rotifer density.

In the figure 3.12 a last simulation using the inner loop is shown. In this case, the use of two different references tries to combine their effects, as in the experiments with the temperature, of a first fast growth and then slowing the population dynamics to keep the density in a range close to the 500 rotifers per millilitre

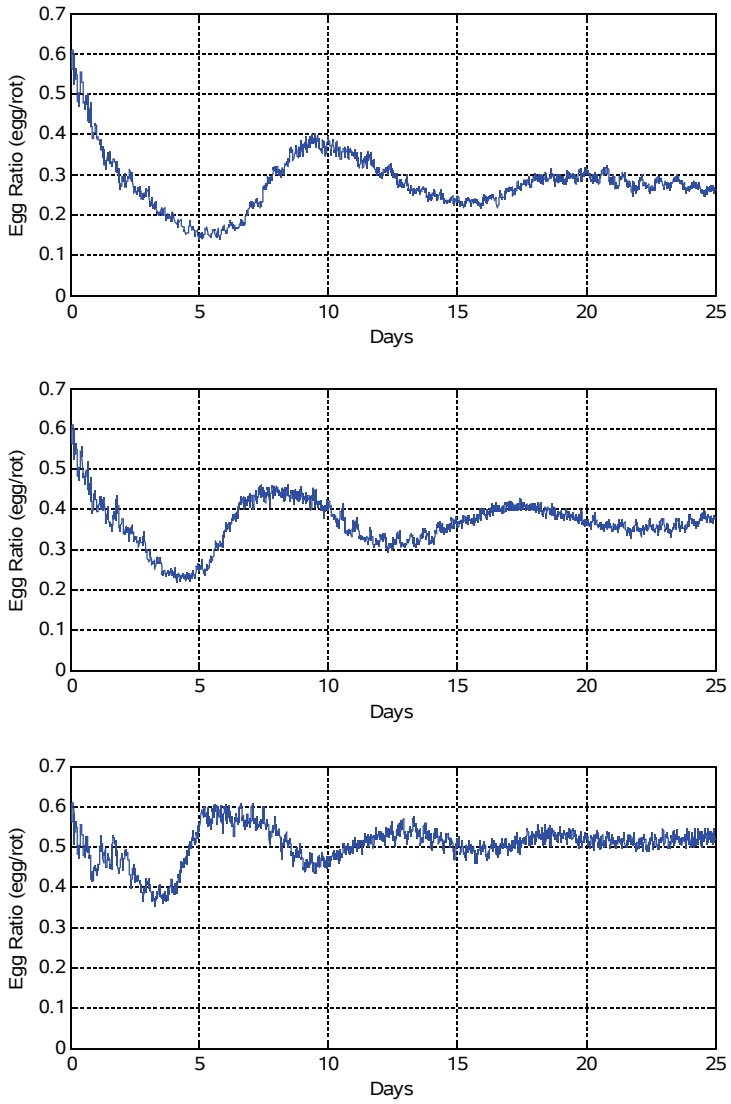

Figure 3.11: Egg Ratios for the simulations with 0.010, 0.013 and 0.024 g/l reference.

For doing that an initial reference of 0.024 g/l is used as the feed density reference, being switched to 0.011 g/l when the density reaches the 300 individuals per millilitre. This value is achieved after about 3 days (one day faster than with a 0.010 g/l litre) and after this point, the population keeps growing and the egg ratio dropping softly. On the sixth day, the 20 % dilution begins and, with an egg ratio close to a 0.3 egg/rot value, the population maintains a very stable level between the 500 and 600 rotifers per millilitre

Of course, depending on the initial state of the rotifer population, these values can vary, taking more time to achieve the 300 rotifers per millilitre density if the initial egg ratio is lower, for example and probably taking a bit longer time to stabilize the egg ratio but, in the end when a stable value of the egg ratio would be reached, and thus also the growth rate, the behaviour would be the same.

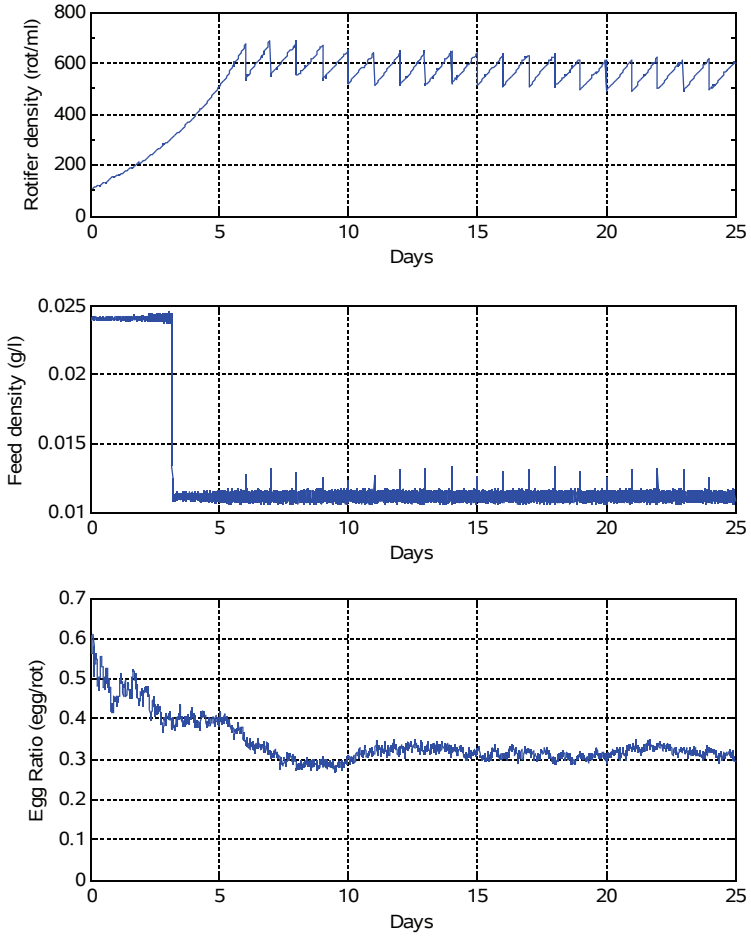

Figure 3.12: Starting ref. of 0.024 g/l, switching to 0.011 g/l when population reaches the 300 ind/ml.

Now, using the complete controller it is possible to obtain a desired rotifer density in the steady state by means of controlling the growth rate. The figure 3.13 shows the results of a simulation in which the density is maintained around a 500 individuals per millilitre For doing that, when the rotifer population reaches the 500 rot/ml the growth rate reference is switched to 0.14, and while the population is under this value, the reference is set to 0.165. As the feed reference is not a fixed value, the egg ratio does not stabilize in a value as happened in the previous simulation, but the effect is that the population oscillates in a small range around the desired value.

The result of the simulation can seem worse than the result when switching from 0.024 g/l to 0.011 g/l using only the inner loop but, 0.011 g/l is the value that seems to stabilize the growth of the population under the specific conditions of the experiment (25 ºC and 20% dilution) and if these conditions are modified the feed reference at which that happens is different. On the other hand, a controller that modifies the feed reference as needed can be used with different conditions as it will try to achieve the feed density needed to get a growth rate independently of the other conditions.

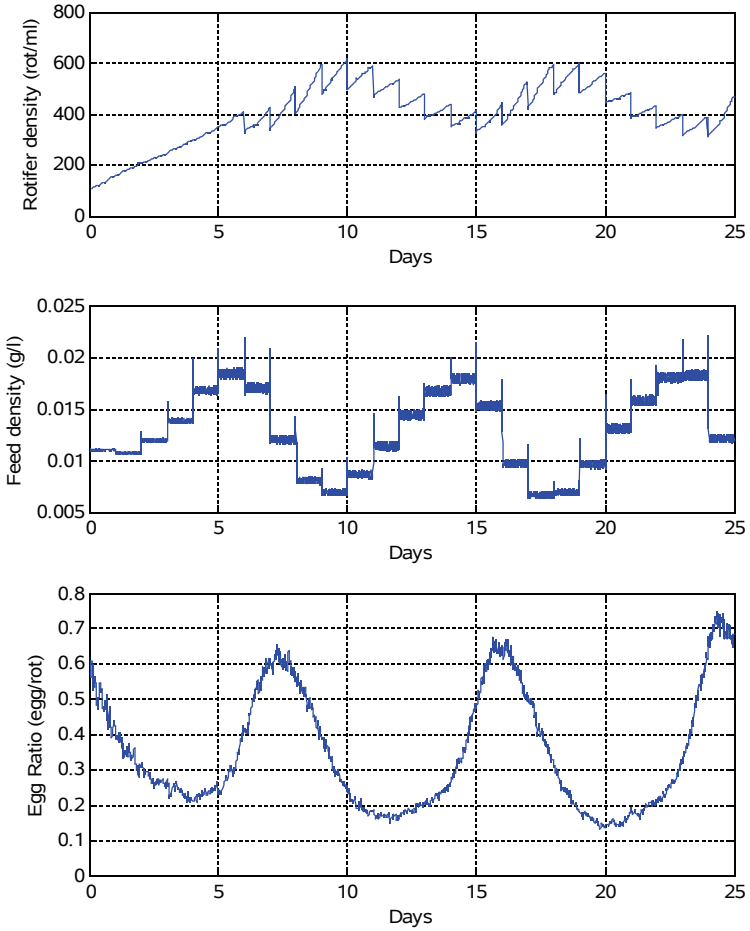

Figure 3.13: Growth rate controller results for a 500 rot/ml density in the steady state.

3.4 Egg Ratio controller

This controller has been run not only with simulations with the model, which were first run for tuning the controller and having some previous data that could be useful when running an experiment with a real rotifer population, but also a 12 day experiment run in a 250 litre tank. The target during these experiments was, as an initial stage of the controller, try to keep the egg ratio of the population in a value, that is, the reference.

During the simulations it was seen that it was better to feed the rotifers during the first three days with just a fixed specific food ration (SFR) and not modify it with any control action to avoid an excessive feed density during the first days, which lead to an also excessive egg ratio value, making it taking longer time to maintain it near the reference and also more oscillating (as the egg ratio is already a parameter which values will be oscillating).

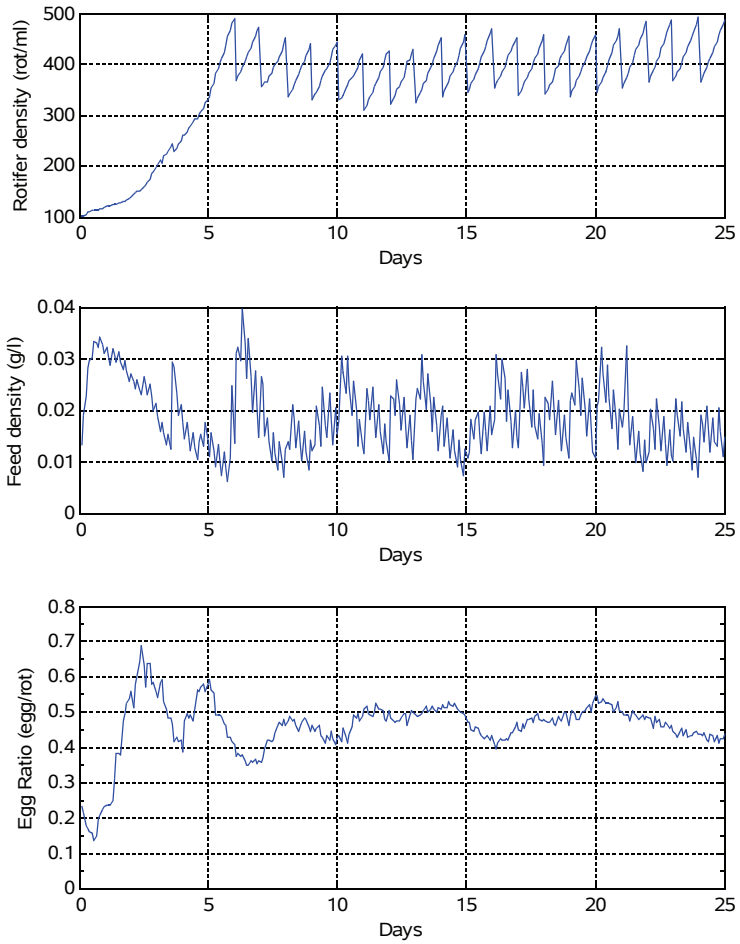

Figure 3.14: Simulation results with a 0.45 egg/rot reference.

In the above figure the results of a simulation with an egg ratio reference of 0.45 are showed. As can be seen, after the first days the egg ratio is always in values close to the 0.45 desired which, if combined with an adequate dilution rate makes the rotifer density stabilize as explained before. In this simulation the volume of the tank was of 200 litres and the dilution rate was set to a 27% of the volume starting on the sixth day. The amount of food used is 11.0746 grams per litre after the 25 days.

After the simulations were done, the experiment using a 250 litre tank and of 12 days length was run where a 0.45 egg/rot reference was used. The initial rotifer density and egg ratio were 108 rot/ml and 0.185 egg/rot respectively, and the temperature measured was 18.3 ºC. Then, a reservoir with 40 g of yeast and 4 ml of rotifer diet was prepared and fed during the next 24 hours. After the first day a rotifer population of 144 rot/ml was measured, what probably means the the initial population was a bit higher than the 108 rot/ml measured (and also the new measure somewhat higher than the real value); and the most probable cause is that the rotifers, taken from another tank, suffered a small temperature shock when set in colder water, making have a lower measure than the actual value. The new egg ratio was 0.29166, and the new rotifer density gave a value of 90 g of yeast for the next 24 hours (during the first days, only the rotifer density is taken into account for the feeding, using a fixed SFR), with the addition of 9 ml of rotifer diet. After that first day the rearing conditions were very good, being all four measured parameters around their optimal ranges. The temperature was 24.0 ºC, the oxygen concentration of 7.0 mg/l, a pH of 7.5 and a salinity of 19

ppt.

At the end of the second day the new rotifer density was 146 rot/ml and the egg ratio reached the 0.71484 egg/rot. The value of the rotifer density indicates that, as mentioned above, the measurement after the first day was a bit higher than the real value but, in this experiment the most important value is the egg ratio and that is less inaccurate than the manual count of rotifers, as being calculated as the number of eggs divided into the number of rotifers, the proportion of rotifer carrying eggs is not dependent on the number of them. The culture conditions continued in good values, 23.3 ºC, 6.4 mg/l of oxygen, pH of 7.5 and a salinity of 20 ppt. As the number of rotifers was the same than the day before, the same happened with the feed and 90 g of yeast plus 9 ml of rotifer diet were set in the reservoir for the next 24 hours.

The next day the rotifer count was of 225 rot/ml and the egg ratio 0.40299 egg/rot. If the formula for obtaining the net growth rate is used with a mortality of 0.1 day^{-1}, with the data from the day before:

$$\mu_{net} = 0.9 \cdot 0.71484 - 0.1 = 0.543356$$

$$rot\_den = rot\_den_{k-1} \cdot (1 + \mu_{net}) = 225\, rot/ml$$

Of course the exact agreement between the measured value and the value estimated is just a coincidence, as long as the values used are not perfectly accurate, but indicates that the measures obtained are reasonably close to the real values, that would be of the same order than the measurements. The new values of the state of the culture: 23.4 ºC, 6.2 mg/l of oxygen, pH 7.5 and the salinity, 19 ppt. And now, after the third day both data, rotifer density and egg ratio are used to calculate the new feed, obtaining an SFR value from the egg ratio and using the PID controller; with all of this the new feed is 272.4 g of yeast plus 27 ml of rotifer diet, that are fed during the next 24 hours.

The values at the end of the fourth day were 334 rot/ml and 0.39735 egg/rot, which led to a 237.6 g of yeast and 24 ml of algae addition. Regarding to the culture conditions all the parameters showed similar values, with the exception of the dissolved oxygen, which showed a 5.0 mg/l. This value was not worrying as it was far enough from the 2 mg/l or less that could be dangerous for the rotifers, but showed that the increase of the population and food added into the tank were reducing the amount of oxygen and that in short time the dilution would be necessary to renew the water and provide more oxygen to the culture, as no oxygen was being added at all.

After the fifth day of the experiment the population reached the 426 rot/ml and the egg ratio was of 0.58696 egg/rot. Again, no special differences between the culture condition values except the oxygen, that now was in a concentration of 3.4 mg/l, making necessary the dilution at the next day in any case. Anyway, the starting of the dilutions was programmed for the sixth day, so the scheduling was not affected, but if it had not been scheduled for that day, the dilution had have to start anyway. The feed used was 382.44 g of yeast and 38 ml of rotifer diet.

The sixth day, the population reached the 646 rot/ml and the culture conditions were: 23.8 ºC, 2.3 mg/l of oxygen, pH 7.5 and 18 ppt salinity before the dilution was done. After the dilution of 30 % (a 30 % of the volume was extracted, 75 litres, and replaced with fresh water) the new values were: 454 rot/ml, 22.0 ºC, 4.9 mg/l of oxygen, pH 7.5 and 18 ppt salinity. The egg ratio, which is not affected by the dilution, was 0.46656 egg/rot. The new feed being 70 g of yeast plus 7 ml of rotifer diet.

The population reached again a 646 rot/ml value after the seventh day, but this time the egg

ratio descended to a 0.1985 value. This initial large variations of the egg ratio were expected to happen after the simulations, so if reduced later it seemed normal and nothing to worry about. The temperature was 24.7 ºC, the oxygen concentration of 4.4 mg/l and pH and salinity remained in the same values measured the day before, pH 7.5 and 18 ppt salinity. After a new dilution the rotifer density was 452 rot/ml, the new temperature was 22.5 ºC, and the oxygen and the salinity increased their values to 5.8 mg/l and 19 ppt, while the pH remained constant. The feed was 654 g of yeast and 65 ml of rotifer diet.

The eighth day, before diluting the new rotifer density was 510 rot/ml and the dissolved oxygen dropped up to a 1.2 mg/l value, which was dangerous for the population if maintained for longer time and caused certainly for the addition of so much food during the last day. The egg ratio was 0.49688 egg/rot and after the dilution 357 rot/ml and 4.3 mg/l of oxygen were measured, returning the parameters to safe values. Then 321.24 g of yeast and 32 ml rotifer diet were set in the reservoir with 10 litres of water.

The next day the following values were measured before the dilution: 566 rot/ml, 24.9 ºC, 1.7 mg/l of oxygen, pH 7.5 and a salinity of 19 ppt. And after diluting the rotifer density was left at 396 rot/ml, while the other parameters were 22.0 ºC, 5.0 mg/l of oxygen, pH 7.5 and salinity 20 ppt. That day the egg ratio was 0.60071 egg/rot and the feed used was 209.4 g of yeast and 21 ml of rotifer diet.

At the tenth day of the experiment a 661 rot/ml density was measured and 3.7 mg/l of oxygen before the dilution, with an egg ratio value of 0.353985. After the dilution the rotifer density was of 462 rot/ml and the dissolved oxygen, 5.8 mg/l. After what happened after the seventh day, when the situation was similar the differential error (the difference between the error and its previous value) was saturated with a value of 0.12 and also the K_d was reduced from the 0.21 value it had to 0.12, that way, preventing an excessive decrease of the oxygen as happened in the eighth and ninth days. With these new values, the feed used was 402.32 g of yeast plus 40 ml of rotifer diet.

The eleventh day 564 rot/ml were measured before the dilution, with a dissolved oxygen of 2.8 mg/l, a low value but beyond the 2 mg/l beginning to be dangerous and far from the 1.2 mg/l registered after the eighth day when the largest amount of food was added. That was the desired effect of reducing the derivative action in the controller and worked correctly. The oxygen concentration was 5.3 mg/l and the rotifer density was 394 rot/ml after the dilution, and with the 0.50733 egg/rot egg ratio, the amount of food for the last 24 hours of experiment was 164.64 g of yeast and 16 ml of rotifer diet.

Finally, at the end of the twelfth day the rotifer density obtained was 432 rot/ml and an egg ratio of 0.34552 egg/rot. In the table 3.1 there is a table with a resume of the values obtained during the experiment, and the figure 3.15 shows the evolution of the rotifer density and egg ratio.

Time (days)		Egg Ratio (egg/rot)	Rotifer density (rot/ml)	Temperature (ºC)	O_2 (mg/l)	pH	Salinity (ppt)
0	BD	0.185	108	18.3	7.3	7.5	19
	AD						
1	BD	0.29166	144	24.0	7.0	7.5	19
	AD						
2	BD	0.71484	146	23.3	6.4	7.5	20
	AD						
3	BD	0.40299	225	23.4	6.2	7.5	19
	AD						
4	BD	0.39735	334	23.6	5.0	7.5	19
	AD						
5	BD	0.58696	426	23.7	3.4	7.5	18
	AD						
6	BD	0.46656	646	23.8	2.3	7.5	18
	AD		454	22.0	4.9	7.5	18
7	BD	0.1985	646	24.7	4.4	7.5	18
	AD		452	22.5	5.8	7.5	19
8	BD	0.49688	510	24.6	1.2	7.5	19
	AD		357	21.8	4.3	7.5	19
9	BD	0.60071	566	24.9	1.7	7.5	19
	AD		396	22.0	5.0	7.5	20
10	BD	0.353985	661	24.9	3.7	7.5	19
	AD		462	22.0	5.8	7.5	20
11	BD	0.50733	564	24.6	2.8	7.5	20
	AD		394	21.9	5.3	7.5	20
12	BD	0.34552	432	24.6	4.7	7.5	20
	AD						

Table 3.1: Resume of the measured data during the experiment with 0.45 egg/rot reference.

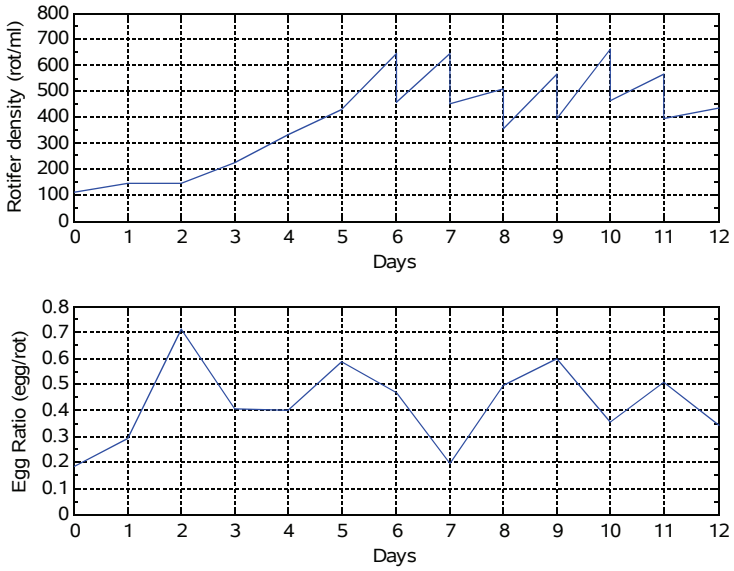

Figure 3.15: Rotifer density and Egg Ratio of the experiment with 0.45 egg/rot reference.

The results show that the controller is able to make the egg ratio to approximate the reference value used, though the length of the experiment does not permit if the convergence to the reference is better in the following days, as it would be expected looking at the simulations. At the end of the experiment 2935.2 grams of yeast were used, 11.7408 g/l. Some differences can be observed with respect to the simulations that are without any doubt due to the difference between the model and a real population and also due to the fact that the way of feeding the rotifers during the simulations and the experiment were slightly different; once every half an hour in the former, and continuously in the latter. It might be possible that when using the continuous feeding the amount of food should be reduced, reducing a bit the oscillation of the egg ratio value, modifying the values of the parameters of the controller (in fact at the end, as explained, the derivative action was reduced) and then the response would be more similar to the one in the simulations. Another source of discrepancies between the simulations and the experiment is that the measurements were done manually (not even with an automated system) while during the simulations these values were extracted from the model itself, having a kind of ideal sensor with exact measurements.

Chapter 4

Discussion

Temperature is a factor that can provide some benefits, letting modify the dynamics of the population and getting a higher or lower growth rate as desired. Studying and implementing a control system that allows to set the temperature to a desired value during the whole cultivation process could be of great interest. On the other hand, that kind of control can be difficult to obtain, specially when talking about the big tanks with thousands of litres of water, where it could be more interesting as higher amounts of rotifers are being produced. And of course, a control like this would have a cost of energy that should be studied for deciding if the benefits from the control are enough to have these expenses or not.

Turbidity appeared to be the obvious way for measuring the amount of food dissolved in the water but, after the several experiments performed, it showed not to be an easy way for getting these measurements, at least when talking about so low concentrations of food. It might be possible to use other turbidity sensors, such as the *InPro®8400*, with different characteristics:

- forward scatter sensor,
- colour compensation (colour affected the measurements in the experiments),
- measuring range from 0 FTU to 400 FTU

  0.0 to 1.0 g/l
- accuracy and reproducibility ⩽1 % ,
- resolution of 0.01 FTU.

But it is also possible that the measurements would be not useful (or at least directly) due to possible perturbations introduced by the rotifers themselves (though having their value probably it could be compensated) and the apparition of ciliates and bacteria, for example. So it should be reconsidered if it is an appropriate way of measuring the feed density inside the tank.

On the other hand, a control based on the control of the feed density seems to have very interesting results, allowing to modify the egg ratio, and thus the growth rate and population dynamics, permitting to adapt the production to the actual needs and making a better use of the resources. In this strategy the inner loop (PID + FF) is not intended to have a direct effect over the dynamics of the rotifer population, but just to follow the feed reference set by the outer loop. This outer loop is the one that, with its control law, will define the dynamics of the population modifying the feed reference. Once the inner loop seems, according to the simulations, to work fine the outer

loop is the one with more interest from the control point of view. In this work a switching law with a proportional controller has been used as a first approach to the problem. Even if not being the best solution (taking into account the nonlinear behaviour of the system) but a first approach, it showed that worked reasonably well and that a further study of this loop could improve the results if the problems regarding to the measurements required are solved.

The control not based on the feed density control has some advantages as for example that does not need a controller running every minute, making the requirements of the hardware lower and cheaper. As seen in the experiment, the controller works moderately well having into account its very initial stage of development. If improved based on the data from this and other experiments, if possible with a length of three weeks, it seems a strategy that can work very well and with a reduced cost of food compared with the amount used in this experiment.

Both of the strategies seem good approaches to the growth control of the rotifers according to the results obtained from the simulations. But the one based on the egg ratio could be applied faster as no new measures are required and, even if it was not possible to make use of it, an automated way of measuring the rotifer density is already developed, with a better implementation of the controller and some more experimental data could be ready to use in not a long time. The biggest problem for the full automation of the process seems to be the measurement of the egg ratio, as an approach with image processing as in the case of the rotifers seems complicated due to their even more reduced size, which makes very difficult that a camera could distinguish them.

Chapter 5

Concluding remarks

The process of cultivating rotifers is nowadays done almost manually, making the process very dependent on the people rearing them, and increasing the probability of a crash when the most experienced of them are absent. Applying control techniques will make the process not only more independent to people and reliable, but also more flexible, being possible to adjust the dynamics of the population to the actual needs for example, as it has been shown in this work.

In this thesis, a first study of the biology and conditions for rearing rotifers has been done and then, applications of cybernetic methods for the cultivation of rotifers have been introduced, aiming mainly to the automation of the process, and more specifically to the growth and dynamics of the population during the cultivation process. Two control strategies have been developed and a method for knowing the feed density in the cultivation tanks through the turbidity of the water has been tried, but due to the negative results it was not possible to make use of it, making impossible to experiment one of the control strategies, which in fact was the main strategy intended to be studied during this work. Due to that, most of the time after the initial study was used for tuning the feed controller running several simulations and trying to get better results with the turbidity sensors, including a delay on the delivery of one of them that delayed all the process. All that left little time to develop, simulate and experiment the new strategy. During the experiment the rotifer counter was intended to use but there seemed to be a problem with the software and as little time was available its use was discarded this time and a manual counting was performed instead, as mentioned before.

The results show that these controllers and, in general, the application of control engineering techniques are worth of further study as they will be able to improve the rotifer production.

5.1 Further work

Several things should be addressed in the future work with these strategies:

- Study the viability of developing a device for the egg counting and/or an observer to reduce the manual operations in the process.
- Solve the problems of the rotifer counter in order to use it in future experiments, having automated and more accurate measurements.

- Study new control laws for the egg ratio controller. Two interesting options could be the use of a Linear Quadratic regulator where the difference between the egg ratio an its reference should be minimized, and the amount of food used bounded to constraints to try to reduce its use; and the use of a predictive controller, such as a GPC or MPC, due to the fact that the amount of food added affects the population during several days, not only during the next one.
- Another control loop could be implemented in the egg ratio strategy to modify the reference, as in the feed strategy, in order to have a higher control of the growth.
- Study if the turbidity could definitely provide a feed density measurement or if an alternative is needed, including the possibility of using an observer.
- Implement new control laws for the outer loop in the feed strategy, depending on the needs of the production, for having the possibility of a more flexible production, with a high growth, a maintenance state, keep a fixed density, etc, and minimizing the use of food in any case.

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Appendix A

Code of the controllers

A.1 Feed density strategy

```
import deb.BaseFeedDynamic;
import deb.Parameters;
import deb.Population;
import deb.Simulator;
import deb.control.Controller;
import deb.fishtank.RotiferPopulation;

public class MyController implements Controller {

	private double ref, base;
	private double Kp, Ki, Kd;
	private double int_e, ek1, sat_e;
	private boolean first;
	private int gain;
	private double dz;
	private double total;

	private double referenceLastRun;
	private double referenceInterval;
	private double growth_ref;
	private boolean r_first_up;
	private boolean r_first_down;

	public MyController(){
		ref = 0.01300;
		Kp = 1.75;
		Ki = 0.15;
		Kd = 0.06;
		int_e = 0;
		ek1 = 0;
		sat_e = ref / 8;
		first = true;
		gain = 1;
		dz = 0.000001;
		total = 0;
		base = 0.000625;

		referenceLastRun = 0;
		referenceInterval = 1;
		growth_ref = 0.31;
		r_first_up = true;
		r_first_down = true;
	}
```

```
	public void setParams(double p, double i, double d){
		this.Kp = p;
		this.Ki = i;
		this.Kd = d;
	}

	public void setRef(double r){
		this.ref = r;
		sat_e = this.ref / 8;
	}

	public double getCallInterval() {
		// TODO Auto-generated method stub
		return 0.000625; //0.000625 of a day = 0.9 minutes
	}

	public void makeControlRun(double t, Simulator sim, Population pop, Parameters
envPar) {
		// TODO Auto-generated method stub
		BaseFeedDynamic bfd = pop.getBaseFeedDynamic();
		double feed, feed_c, err, dif_e, u;

		double rot_den = pop.getHerbivoreCount() / envPar.get("V_w0", 0) / 1000;

		RotiferPopulation rotPop = (RotiferPopulation)pop;

//		OUTER LOOP
		if(t-referenceLastRun >= referenceInterval){

			if(rot_den >= 300){
				if(r_first_up){
					growth_ref = 0.14;
					r_first_up = false;
				}
				else if(rot_den >= 500){
					growth_ref = 0.14;
					r_first_down = false;
				}
			}
			else if(rot_den < 500 && !r_first_down){
				growth_ref = 0.165;
			}

			double gr_e = growth_ref - (0.9*rotPop.getEggRate() - 0.16);
			System.out.println("Growth rate error = "+gr_e);
			this.setRef(ref + 0.02*gr_e);
			referenceLastRun = t;
		}

		System.out.println("Density: "+rot_den+" rot/ml");

		//INNER LOOP

		if (rot_den < 100.0 && gain!=1){
			this.setParams(1.75, 0.15, 0.06);
			this.base = 0.000625;
			gain = 1;
		}

		if (rot_den > 100.0 && rot_den < 225.0 && gain!=2){
			this.setParams(1.75, 0.15, 0.06);
			this.base = 0.00100;
			gain = 2;
		}

		if (rot_den > 225.0 && rot_den < 300.0 && gain!=3){
			this.setParams(2.0, 0.18, 0.06);
```

```
                this.base = 0.00150;
                gain = 3;
            }

            if (rot_den > 300.0 && gain!=4){
                this.setParams(2.25, 0.15, 0.06);
                this.base = 0.00150;
//              this.setRef(0.0110);
//              envPar.put("T_w", 22);
                gain = 4;
            }

            System.out.println("Ref: "+ref);

            if(first){
                u = ref;
                first = false;
            }
            else{

                feed = ref*base;

                feed_c = pop.getBaseFeedConcentration();
                System.out.println("Concentration = "+feed_c);

                err = ref - feed_c;
                dif_e = err - ek1;
                ek1 = err;
                int_e = int_e + err;
                if (int_e < -sat_e) int_e = -sat_e;
                else if (int_e > sat_e) int_e = sat_e;
                System.out.println("Accumulated error: "+int_e);

                u = feed + (err*Kp) + (int_e*Ki) + (dif_e*Kd);

            }
            if (u >= dz){
                bfd.increaseLevel(u);
                total = total + u;
                System.out.println(u+" g/l added");
                System.out.println("Total feed added: "+total+" g/l");
            }
            else if (u < 0){
                System.out.println("Let the rotifers it the excess");
            }
        }
}
```

A.2 Egg Ratio controller

```
import deb.BaseFeedDynamic;
import deb.Parameters;
import deb.Population;
import deb.Simulator;
import deb.control.Controller;
import deb.fishtank.RotiferPopulation;

public class er_sfrController implements Controller {

    private double ref, sfr, err;
    private double Kp, Ki, Kd;
    private double int_e, ek1, sat_e, sat_d;
```

```
	private int count;
	private double dz;
	private double total;
	private double ts;
	private double rot_den;
	private boolean first, second, third;

	public er_sfrController(){
//		ref = Egg Ratio reference
		ref = 0.35;
		err=0;

		Kp = 0.006;
		Ki = 0.03;
		Kd = 0.21;
		int_e = 0;
		ek1 = 0;
		sat_e = ref / 6;
		sat_d = 0.12;
		count = 1;
		dz = 0.000001;
		total = 0;
		sfr = 1.8e-006;
		ts = 0.02083; //Ts = 0.5 hour aprox.
		rot_den = 50;
		first = true;
		second = true;
		third = true;
	}

	public void setParams(double p, double i, double d){
		this.Kp = p;
		this.Ki = i;
		this.Kd = d;
	}

	public void setRef(double r){
		this.ref = r;
		sat_e = this.ref / 6;
	}

	public double getCallInterval() {
		// TODO Auto-generated method stub
		return ts;
	}

	public void makeControlRun(double t, Simulator sim, Population pop, Parameters
envPar) {
		// TODO Auto-generated method stub
		BaseFeedDynamic bfd = pop.getBaseFeedDynamic();
		double feed, dif_e, u;

		RotiferPopulation rotPop = (RotiferPopulation)pop;

		if(count == 1){

			rot_den = pop.getHerbivoreCount() / envPar.get("V_w0", 0) / 1000;
			System.out.println("Rotifer density: "+rot_den+" rot/ml");

			double er = rotPop.getEggRate();
			System.out.println("Egg rate: "+er+" egg/rot");
			err = ref - er;

			try{
				sfr = (0.184/(0.537 - 0.9*er))/1000000;
			}catch(Exception e){}
			if(sfr < 0) sfr = 0.74e-006;
			else if(sfr > 1.6e-006) sfr = 1.6e-006;
```

```
                count++;
            }
            else{
                if(count < 49) count++;
                else{
                    count = 1;
                    if(first) first = false;
                    else if(second) second = false;
                    else if(third) third = false;
                }
            }

            if(first || second || third){
                sfr = 1.0e-006;
                feed = sfr*(rot_den*envPar.get("V_w0", 0)*1000)*(0.01*ts);
                u = feed;
            }
            else{
                feed = sfr*(rot_den*envPar.get("V_w0", 0)*1000)*(0.009*ts);

                dif_e = err - ek1;
                if (dif_e < -sat_d) dif_e = -sat_d;
                else if (dif_e > sat_d) dif_e = sat_d;
                ek1 = err;
                int_e = int_e + err;
                if (int_e < -sat_e) int_e = -sat_e;
                else if (int_e > sat_e) int_e = sat_e;
                System.out.println("Accumulated error: "+int_e);

                u = feed + (err*Kp) + (int_e*Ki) + (dif_e*Kd);
            }

            if (u >= dz){
                bfd.increaseLevel(u);
                total = total + u;

                System.out.println(u+" g/l added");
                System.out.println("Total feed added: "+total+" g/l");

            }
            else if (u < 0){
                bfd.increaseLevel(feed);
                total = total + feed;

                System.out.println(feed+" g/l added");
                System.out.println("Total feed added: "+total+" g/l");

            }
        }
}
```