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Empirical investigation of population dynamics of Alona cambouei

A thesis submitted towards partial fulfillment of

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by

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Certificate

This is to certify that this dissertations entitled "Empirical investigation of population dynamics of Alona cambouei" towards the partial fulfillment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research Pune, represents original research carried out by Subhajit Das at Population Biology Lab under the supervision of Dr.Sutirth Dey during the academic year 2010-2011.

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Empirical investigation of population dynamics of Alona cambouei

Abstract- - Understanding the causes and consequences of extinction is an important endeavor of conservation biology. In this study, we investigate the population dynamics of Alona cambouei. Our aim was to try and build a micro-environment for study and maintenance under laboratory conditions. Other than that we also try out a set of experiments to try and understand population dynamics of Alona cambouei. Most of our experiments were carried out on cell culture plates for small scale population studies. Even though we succeed in sustenance of Alona stocks over a long period, the results of the experiments carried out was not significant. The results implied for a choice of different setup all together to carry out our experiments. Future work may focus on study of migration related behavior and under deteriorating conditions on population growth and time to extinction.

Introduction

Extinction is the fate of all population given ample amount of time. Present rate of extinction in nature is much higher than the rate of extinction in the earth's history (Pimm et al.1995). Active management of species is required to maintain healthy ecosystems. It is important to anticipate the effect of human activities such as species over-harvesting and other such disruptions in the nature(Biodiversity & Services; Dudgeon 2010). Thus we need to have a better understanding of the quantitative theory of extinction. The various causes of extinction can be grouped under three major categories, namely demographic factors, genetic effects and environmental factors.

Demographic factors

Variation among individuals has the greatest effect on smaller populations (Lande et al. 2003). Population variability can cause fluctuations in population density, thus affecting the number of individuals. Large populations are more stable to variability and hence are less prone to extinction. On the other hand, a population with greater variability has higher risk of extinction (Vucetich et al 2000).

Migration is a major player in population dynamics as it can result in growth in population if the immigration rate is positive and negative when the population is declining. As migration acts to average out population density between patches, it reduces variability in population size and hinders extinction. Thus by rescue of small population in a meta-population, migration is predicted to increase overall fitness of interconnected populations.

As migration may be helpful in metapopulation persistence, it can also be said that if migration rates between two population is high, population dynamics will be synchronized across sub-populations, hence increasing the chance of simultaneous reduction, where demographic stochasticity can act. Population synchrony may be important in population dynamics across sub-populations, hence increasing the chance of simultaneous reductions to small size where demographic stochasticity can operate (Earn et al. 2000). Another such example is given by Dey and Joshi (Dey and Joshi 2006) in which high, low and no migration studies on *Drosophila* showed that while high migration rate synchronized sub-population fluctuation, low migration rate did not.

Genetic effects

Generally it is seen that most species are driven to extinction by environmental or demographic factors even before genetic factors can act on them (Mace & Lande 2011). One such study done on *Drosophila* saw that population extinction rates were consistently proportional to in-breeding coefficient, the chance of driven to extinction being highest at full-sibling mating populations (*Reed et. al 2003)*. It was also seen that all inbred population had lower fitness than outbred control population. Hence, inbreeding depression along with environmental stress can lead to rapid extinction in some cases.. Genetic diversity can also be maintained by migrating population within a meta-population.

Environmental Effects

Habitat quality is the major player that leads most of population towards extinction. Factors like deteriorating nutrient condition, sudden increase in predator population, migration or addition of a new predator in the trophc cascade or a sudden destruction of ecological niche due to environmental factors are some such static environmental effects. Exposure to deteriorating habitat with limited resource and space can support small populations, hence leading to extinction because of demographic or genetic factors (Klok & De Roos 1998). Similarly variation in food abundance over sub-population increases the probability of extinction and decreases time to extinction. Spatial variation also decreases extinction risk by affecting the synchrony across the spatially segregated sub-populations, reducing the chance of simultaneous reduction of population to small sizes.

We used Alona camboeui collected from the wild for population dynamics studies under lab conditions. Alona cambouei is one of the most robust Cladocerans found in nature (Sinev & Hollwedel 2002). Along with benefits of a asexual reproduction, absence of egg and larval stages makes it a suitable candidate for population dynamics studies. The birth rate for the asexual reproducing population is extremely high and the growth rate does not depend on the number of males and females in the population, as every one is capable of reproducing. The life cycle of organism is in days, and absence of any complex behavior acts as an additional advantage. We seek to understand the various factors that may lead to extinction of Alona populations.

Materials and Methods

Field collection

Sample collection was done in the hot springs of Pali located in Unhere budurk, Kolaba district, state of Maharashtra, India(18 33' 25.20"N,73 13'05.34"E)(Padhye et al.

2010). Water source from there erupts into 3 ~2 X 2 X 2 m tank. And then from here these tanks go to neighboring puddles of tepid water.

Qualitative samples were collected in February, April June of 2009 and also in February and May of 2010. The temperature of these lakes varied between 34.5 to 36.7 with the temperature being in month of June and lowest in February. pH of the water remained very normal with hydrogen ion concentration varying between 7.0 to 7.38.

Stock was maintained in autoclaved pond water and EPA medium was used for experiments. The composition of EPA mixture is $NaHCO_3$ 96 mg/liter, $CaSO_4$ 60 mg/liter, $MgSO_4$ 60 mg/liter and KCL 4 mg/liter. EPA was the obvious choice for Alona medium and it is very widely used for studies on all kinds of cladocerans.

100 ml beakers were used for stock maintenance, while 6,12 and 64 well cell-culture plates were used setting up the population dynamics experiments .The common source of food for both experiments and stock was *Spirulina* suspension .*Spirulina* suspension was prepared from dry *Spirulina* extract capsules available in market.

Stock Maintenance-

100 ml of autoclaved pond water was taken in a beaker. About 60-80 alona were picked from the old stock and re-inoculated into the new beaker. Preference for adult organisms was given while selecting Alona from old stock. Use of the stock was avoided in case of contamination. Spirulina suspension is prepared by weighing ten mg of dried spirulina extract and mixing it with 10 ml of distill water to create a 1mg/ml stock suspension. 800 ul of the spirulina suspension was fed to the stock once in a week .

Experimental setup and Alona transfer

For setting a experiment in cell culture plate ,we used autoclaved plate and washed in alcohol. Appropriate amount of EPA medium i.e. 4 ml for 6 well plates, 8 ml for 12 well plates was for used. One milliliter pipette with extra narrow opening was used in Alona inoculation as they are useful in picking up one Alona at a time. About 10 to 20 individuals were picked and put in single well, after which the pipette was cleaned for contamination using alcohol. The Alona were transferred from the first well to the other using similar technique. This procedure was repeated 2-3 times, transferring fewer and fewer Alona to the adjacent well. A new pipette was used to pick 4-5 Alona along with very little amount of EPA medium put on a slide. Individual Alona were picked using the pipette with the help of microscope. Finally, single Alona were inoculated in different wells as requirement and treatment needed in the experiment.

Initial studies on Alona cambouei were carried out in a 6 well cell culture plates. One Alona for each cell was inoculated in the plates containing autoclaved pond water medium. To begin with they were grown at a very low food concentration of 0.01 mg/ml to 0.1 mg/ml spirulina suspension. Daily counting and monitoring was done.

As the nutrient content and chemical composition of the pond water was unknown we decided to switch to EPA. First set of experiments were carried out to find growth rate of a time at different food concentration. Alona along with studying the effects of high and low temperature on Alona population dynamics. To carry out this experiment 12 well cell culture plates were used. These experiments were carried out taking into consideration that we needed to find out the daily dosage of Spirulina suspension consumed by Alona.

After which experiments were fed on every alternate day and water was changed once in 3 days, by removing 1 ml of medium and adding one fresh ml of EPA medium . This was done to prevent accumulation of toxic metabolites that maybe present in Alona ordure.

The main aim of the various experiments was to understand various factors like growth rate ,time till extinction ,role played by environmental factors like habitat quality and size on extinction. Other than that we also want to test various hypothesis given by their studies on Alona Cambouei. Similar kind of work have been carried out on other Daphnia and Claodoceran species like , Moina (Atienza et al. 2008; Murugan 1989; MURUGAN 1975; Submitted 2010)

Standardization experiments:

1. Food quantity

Initial set of experiments were carried out get an idea about the daily consumption rate of Alona cambouei in EPA medium .As our aim was to carry out extinction experiments on Alona cambouei in which they are kept under deteriorating food concentration the knowledge of daily consumption rate or regular dosage was mandatory. Hence, this experiment was designed on the lines of various extinction related work done by Drake's lab on Daphnia magna. Along with it we were also keen on finding out the varying temperature range under which an Alona can survive in lab conditions. The growth rate at different temperature is also an important factor in understanding Alona extinction .

Alona were grown on three different levels of food concentration. Low (0.0006 to 0.0009 ug/ml ,medium (0.001 to 0.004)and high (0.005 to 0.008). Three replicates of food were taken for each food concentration. Three replicates i.e. three different wells were

used in a column of 6 well cell culture plates. Graph for the results of the first experiment was plotted using the average number of Alona at each food concentration over 18 days. the time-series plot of the experiments is given in Figure.1 .

2. Effects of temperature

Similarly Alona growth rate was seen under two low concentration(0.0006 and 0.0009 ug/ml) and high two high concentration (0.001 and 0.003 ug/ml).Three replicates of each concentration was used .Average number of Alona for each concentration was plotted in time series plot over 18 days. The results are given in figure 2.

3. **Antifungal agents**

As natural pond water contains its own anti fungal reagents these property may be due to presence of various actinomycetes (Keast and Tonkin 1983, Nasser M. El-Banna 2007) or plant extract (Binns S & Correa C 1992). Hence the rate of fungal infection on the experiments done on pond water as well as stock maintenance beakers was comparatively less than the rate of fungal infection of the EPA medium. To prevent fungal growth in the experiments setup with EPA in them we tried out a set of anti-fungal reagents. Most commonly used anti fungal reagents are imadizole and benzoate. The azole anti fungal agents are the largest class of synthetic antimycotics. Azole anti-fungal agents inhibit lanosterol demethylase in yeast and fungi. This enzyme converts lanosterol to ergosterol which is bio regulator of fungal cell integrity and fluidity. While Benzoate at higher concentrations enters the yeast cell in the undissociated form, and its neutralization within the cell can cause a shift of the pH of the intracellular level. Daphnia Toxicity level of Imadizole for Daphnia was EC50: 341.5 mg/l /48 h(AppliChem MSDS A6615) and action of Benzoate demonstrated to be pH dependent, with a lower 24-h EC50 (102 mg/liter) at acidic pH (Concise International Chemical Assessment Benzoate 2005). Thus to find out

the amount of anti fungal reagent required for single Alona to survive we tested both the reagents at concentration varying between 15 to 250 mg/liter .As an adult Alona is much small in size as compared to a Daphnia adult we chose very low levels of anti fungal reagents .12 well one Alona each well setup was used to carry out the experiment.

Imadizole seem to be the most useful anti fungal reagent for the use in Alona related studies. Benzoate Seemed to be more lethal for Alona as at all concentration i.e. varying from the concentration of 15 to 250 mg/liter .All of the Alona turned white as Benzoate had penetrated their cell wall and resulted in high mortality .Performance in Imadizole was not good enough at concentration above 150 mg/lire ,but the optimal concentration was between 15 to 25 mg/lire .As Imadizole is bio-degradable ,hence Imadizole was put in stock as well as experiment periodically at above mentioned concentrations.

Extinction experiments:

Next set of studies was done on the lines of extinction studies done by Drake's lab (Drake & Griffen 2009; Griffen & Drake 2008a; 2009a;Griffen, B. D., & Drake, J. M. 2008a). In this experiment, every well of a standard 12 well cell culture plate containing four ml EPA medium with one *Alona* each, were fed with 25 or 50 micro liters of 100 micrograms per ml of *Spirulina* suspension on every alternate day. The population was monitored every day and counted every alternate day. After satisfactory growth had occurred and the population appeared to be doing well, from that point onwards the feed administered was reduced every alternate day by 2 and 4 micro liters per feed. An insulin syringe was used to remove 1 ml medium and replace with fresh autoclaved medium every week. This was done to prevent waste accumulation. The results are presented in figure 3.

Results and Discussion

As Alona cambouei were being used for the first time for population dynamics studies, we began with investigating the basic behavioral patterns of Alona .Most of the Alona were photo phobic while some of them also showed opposite behavior growth and survival was better in stocks as compared to experiment, where high concentration of biomass excrete was present. They molt 3 to 4 times before till they attain the gravid female stage.

Result of the initial studies was used to get an idea of Alona for stock maintenance and design of other experiments. The Alona count was not possible beyond 40-50 individuals per well, as their movement from one side to other makes it difficult to do an exact count of Alona. This data and observation was helpful in setting up the right conditions and feeding rate for the stock. Re-inoculation of alona in the well was done where Alona died within 1-2 days of setting the experiment. One of the important observations was that even though the adult Alona might be dead within 1 day of inoculation, but the juvenile and new born Alona were still present in the well. As these juvenile or new born Alona have a significant size difference as compared to a gravid adult Alona, hence we found that even though adult Alona might be dead in the well but still there is a high probability of finding a juvenile Alona in the well.

One more important observation was regarding the presence of Alona in the brood pouch of gravid female as the new born adult were seen to be coming out of the brood pouch of a dead Alona .As the probability of picking one adult Alona but more than one

juvenile Alona was high, and the size difference was highly noticeable .The use of 1 ml pipette resulted in picking up various juvenile Alona along with one adult.

First experiment (fig 1) was run for 18 days after which observation was stopped because of high mortality rate in most of the wells. At low food levels the performance was very poor ,the highest mean achieved was at concentration of 0.0009 which went to a maximum of 3 Alona in one of the wells. This distribution may be attributed to the fact that low food concentration may act as a stress for Alona hence leading to high mortality rate.

In case of high and medium food concentration ,the performance was better. The highest mean population for high and medium treatment was 4 and 7 at 0.003 and 0.007 ug/ml concentration respectively. Thus better growth was seen at high food concentration in isolated environments. High food concentration also acts as a positive factor for the juvenile and new born Alona as they grow faster and there comparative mortality rate was low. The number of Alona went down by the 16^{th} day and most of the population had gone extinct by the 18^{th} day. Cases of such behavior may be a result of accumulation of metabolites in the water. Failure to achieve a higher population density in a cell culture plate can be attributed to the various micro nutrients found in pond water but absent in EPA medium.

Effect of temperature can be seen in figure 2. where Alona were kept at a temperature of $18^{o}C$ and $37^{o}C$ respectively. In their natural habitat, the temperature varies over a huge range .Even during a single day the temperature may vary anywhere between $7^{o}C$ to $8^{o}C$.As seen in the graph the mortality was higher and faster at $37^{o}C$.Even under similar concentration mortality rate was higher for $18^{o}C$.This shows that at temperature below optimal range growth rate is much slower and higher temperature is not suitable for

Alona survival. Hence mortality rate must be higher under high temperature. The survival in the pond and lake is possible in contrast to cell plates may be because of the sheer number of the individuals and depth of the two ecosystems. While in pond and lakes the temperature at the surface may vary, but at the bottom of the lake the temperature remains very much constant .Alona escapes to the bottom of the lake under stressful temperature.

In the next experiment the plan was to reduce the feed amounts gradually after we saw good growth in the well, however that did not happen. We saw slow growth rate in the beginning in individual well, soon the population crashed with the total number of *Alona* in a well going up to a maximum of 3 in the case of the populations fed with 25 micro liters and 7 in the case of the populations fed with 50 micro liters. Figure 3 is a time series plot of mean of the 12 wells over 9 days. As we can see from the graph the population average went up to its maximum value of 1.8 Alona/well for 50 ug/ml by the fifth day after which it came crashing done, where as in case of 25 ug/ml the growth was little and the mean was also less than 1.2 individual/well .It corroborates our earlier result that high concentration is required in the beginning of a new population to grow and multiply. The reason of a sudden crash of population is unknown.

We repeated the same set up again with increased feed of 50 and 100 micro liters, but no changes were observed, the populations crashed within seven days. In one of the twelve well plates we had inoculated five *Alona* in each well. Though they still crashed but their survival rate was longer in this experiment compared to the other setups in which only one *Alona* was inoculated per well.

Thus, we were not able to sustain the cultures for long enough period to obtain meaningful data that can be subjected to statistical analysis. All simple reasons like lack of food and

fungal contamination was ruled out and we came to the conclusion that maintaining the cultures in cell culture plates is not practicable. While we were not able to figure out the precise reason for this, we suspect that space could have been a major constraint. Hence, the idea of using cell culture plates for population dynamics studies of Alona was dropped completely and other alternatives were tried for. Taking example from such experiments in *Daphnia* bigger vials like 50ml cell culture bottles could be used. Other than that experiment can be conducted in small laboratory microcosms 31.5x21.7x1 plates made out of Plexiglas with 700mlto 1400 ml water capacity in them. With 24 hours fluorescent light and the temperature held constant at 23°C.

Further studies can be carried out on migration of Alona cambouei from one sub-population to other. A prototype of interconnected wells within a cell culture plates was developed to study migration from one patch to other. Migration can be natural when the patches are inter connected to each via a canal, or this migration rate can be fully controlled and desired number of organism can be added to different wells or sub-populations.

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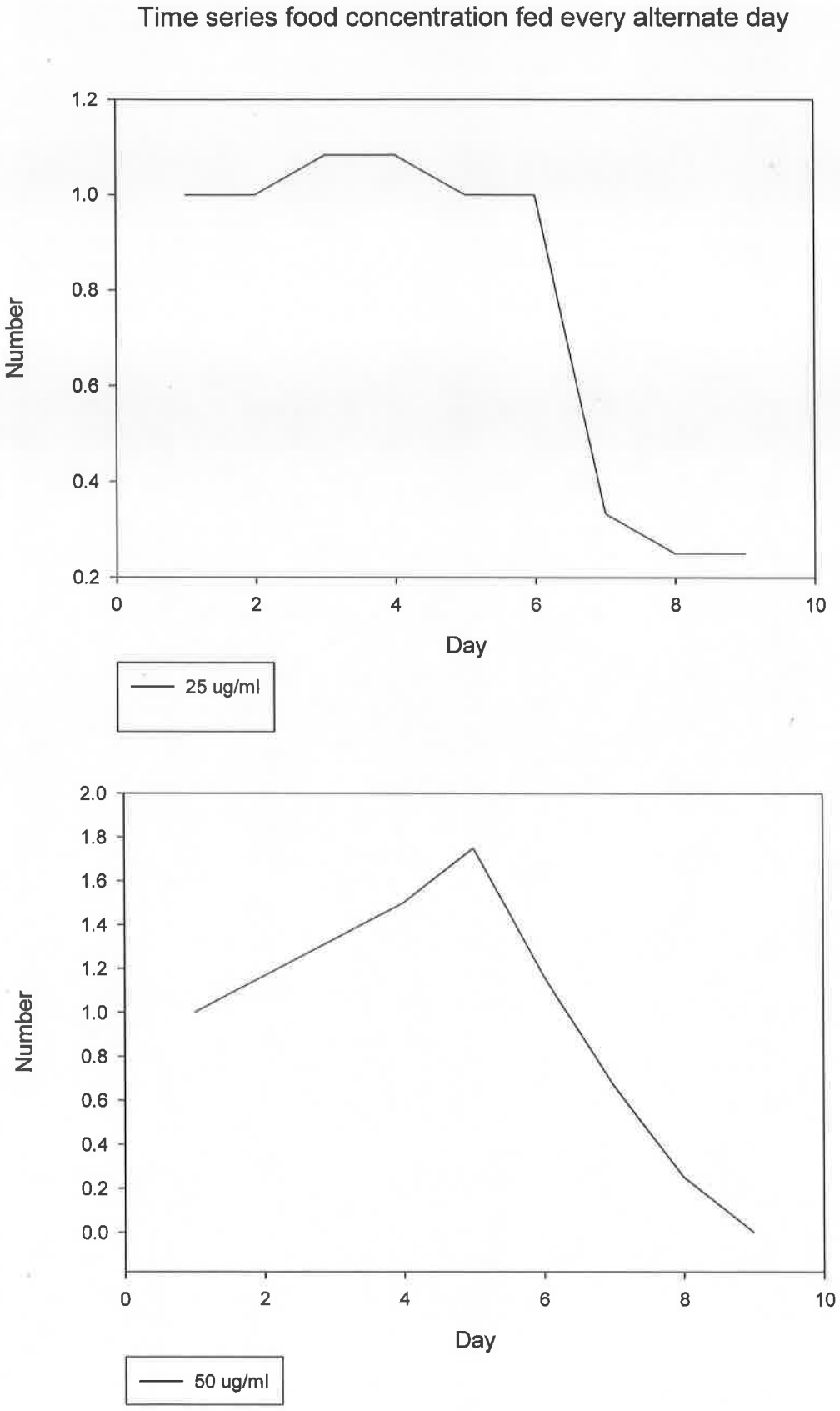

Figure 3.Time series plot for Alona fed with 25 ug/ml and 50 ug/ml every alternate day.

Figures

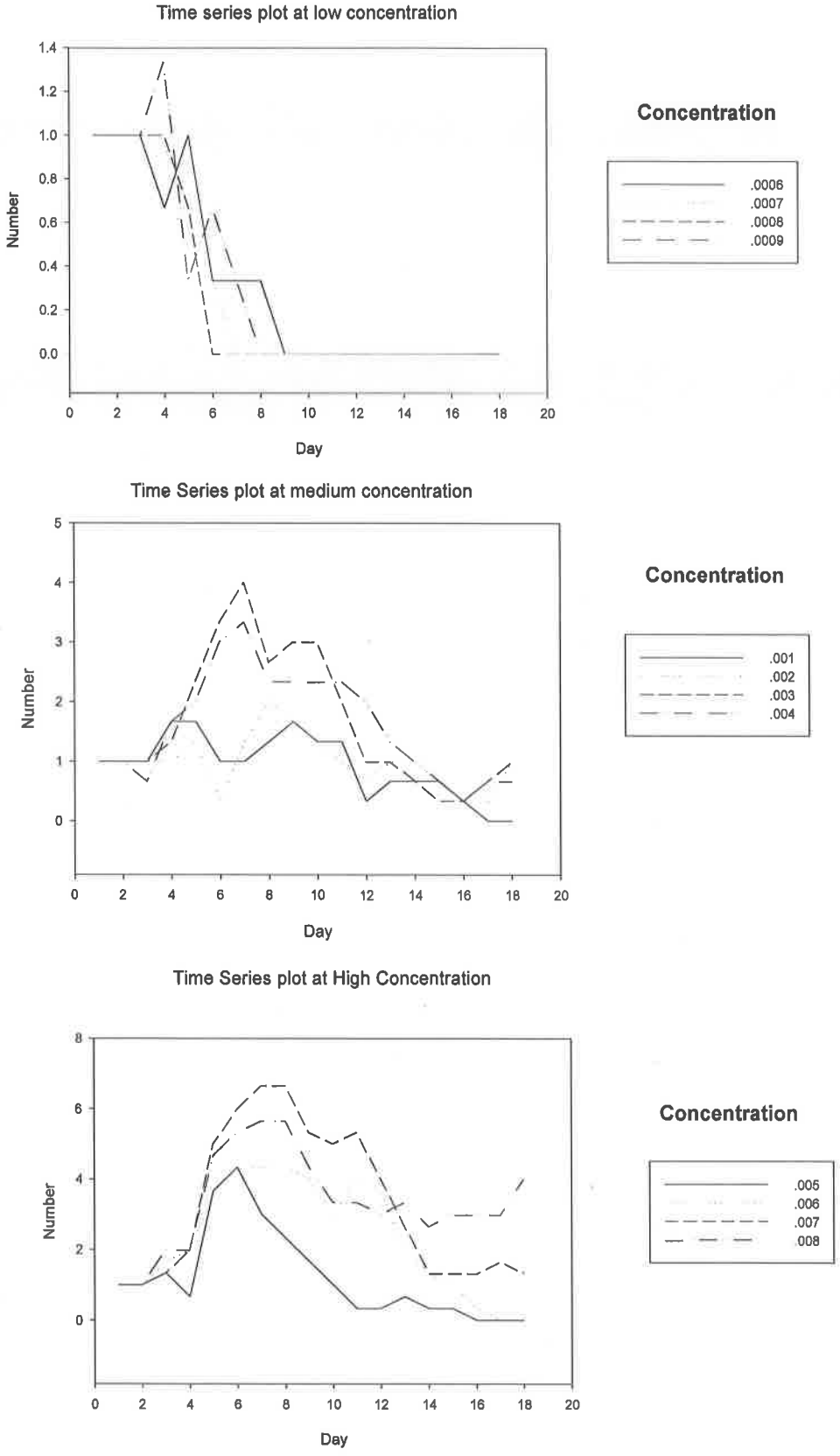

Figure 1.Time series plot of Alona at 25^{O}C low,medium and high food concentration.

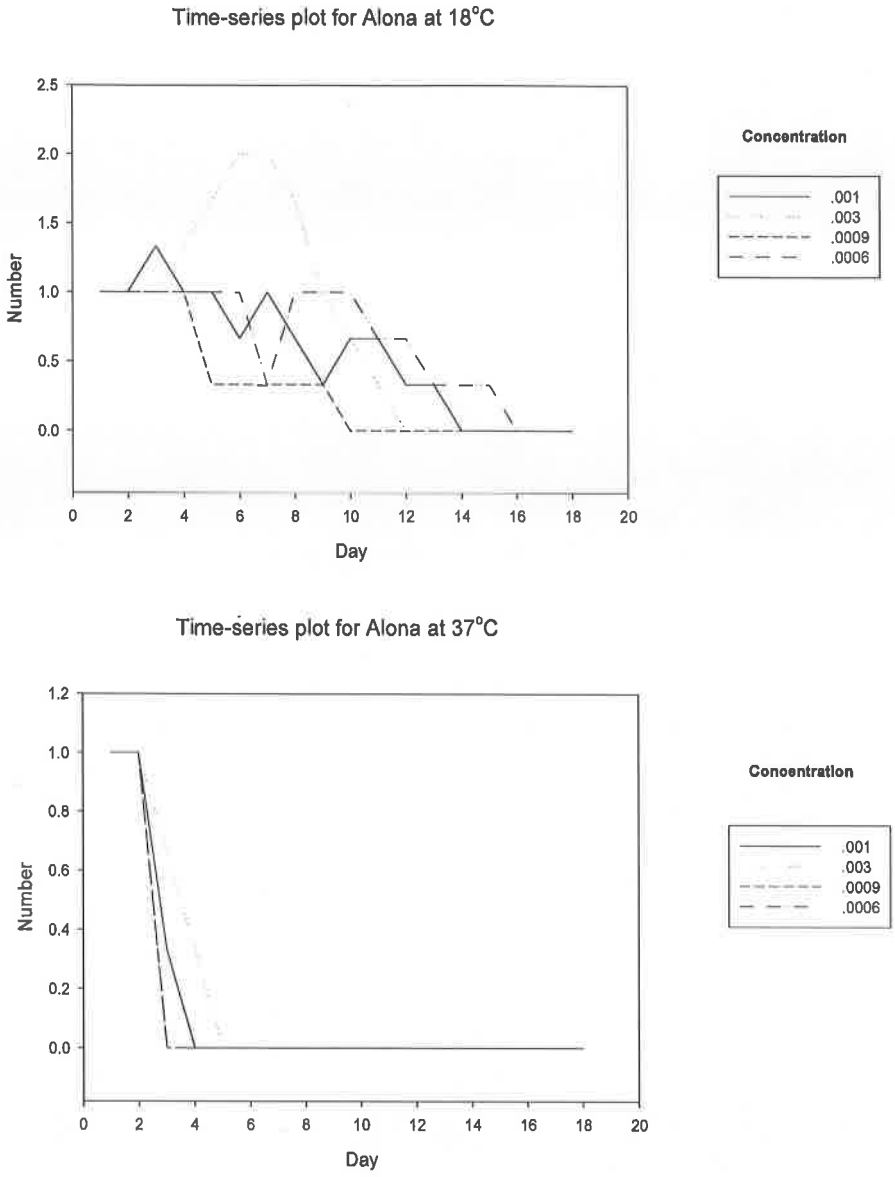

Figure 2.Time series plot for Alona at $18^{O}C$ and $37^{O}C$