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Measurements Population Growth and Fecundity of Daphnia Magna to Different Levels of Nutrients Under Stress Conditions

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1. Introduction

In nature, zooplankton is the main nutritional source of poslarvae and young fish. The natural food offers essential nutrients to guarantee the survival and the growth of fish during their first development stages (Furuya et al. 1999). The description of feed value of living food, has been made by Watanabeetet al., (1998); Kraul, (2006). Living food has a vital job on seed production in fish farms. Without this living food, it is not possible to overcome an adequate survival rate, in species exclusively dependent (Kubitza, 1997; Lahnsteiner et al., 2009).

Micro crustaceans are highly important in aquaculture, mainly the freshwater genera *Moina* and *Daphnia spp,* these two are found in diverse natural environments (FAO, 1996).*Daphnia* genera includes *D. magna, D. pulex, D. longispina* among others. In crops of freshwater spices, poslarvae are fed with 2 or 3 organisms during the beginning of their first hexogen feeding, during their first 10 to 30 days (Lubzens & Zmora, (2003), as cited in Stottrup & Mc Evoy, 2003; Botero, 2004; Prieto, 2006). It is evident the importance of *D. magna* as live food. Authors such as Emmens, (1984), have been reported that *Daphnia spp.* is the best foodstuff for tropical fishes, frequently used food source in the freshwater larviculture (i.e. for different carp species) and in the ornamental fish industry (i.e. guppies, sword tails, black mollies and plattys etc.) (Delbare & Dhert, as cited in Lavens & Sorgeloos, 1996), native and foreign species, for example White Cachama *(Piaractusbrachypomus),* Black Cachama, *(Colosoma macropomum),* Bocachico *(Prochilodus magdalenae),* Yamú *(Brycon amazonicus),* Sabaleta *(Brycon henni),* Dorada *(Brycon moreii),* Striped Bagre *(Pseudoplatystoma fasciatum),* Pacu *(Piaractus mesopotamicus),* among others (Botero; 2004; Prieto, 2008).

It is known of certain difficulties on live food production, due to the fluctuations on the natural conditions of aquatic environments and the elevated infrastructure requirements, equipment, maintenance spending and working labor. It is not possible to produce a constant amount of live food in a regular basis (Kanasawa, 2000). On the other hand the laboratory research to develop culture techniques *of Daphnia magna* has been widely studied, this is because it is easy to cultivate and has a low cost in high densities. Additionally their maintenance in a small space makes them an economically viable alternative culture (Terra, et al., 2010).

The secondary production in lakes is supported by zooplankton, zoobenthos and fish; this means that this group is diverse from the taxonomic and functional point of view. On scale work of authors such as Stotz and Pérez (1992) and Andrade et al., (2009), emphasizes the necessity to recognize the production of a secondary source to determine variables such as maximum extraction. In fact secondary extraction is considered as one of the most important parameters to evaluate the population utilization sustainability (Andrade et al., 2009).This section presents production secondary variables, following the method designed by González, (1988) that is specific for the cladocerans, showing the results obtained at the laboratory scale.

This chapter presents an experimental evaluation of two *Daphnia magna* populations, the first population integrated by neonates and the other by adults in early reproductive stage under stress conditions. This stress condition on the test was made by using 3 cm^3 multi-cells, on each treatment, under controlled conditions of room temperature (21 – 25 ˚C), water temperature (22 – 23 ˚C) and pH (7.6). The diet used was *Saccharomyces cereviseae*, potatoes (*Solanum tuberosum*) and a fatty acid enriched environment n-6 (soy oatmeal). The diet and enrichment concentrations were 30 ppm and 15 ppm, factorial arrangement of 2^3, in concentrations of 15 and 30 ppm mixture of nutrients: yeast and potato and the same concentration for enrichment. Four replicas/treatments were made (32). The Feeding was on a daily basis for 20 days to determine the population performance effect. The productive variables were evaluated: maximum density (Dmáx) daily average density (Dmd), doubling time (Td),specific growth rate (k), performance (r) , numeric growth (PN), birth rate (b), (Edmodson equation), individuals average number ($\bar{N}$), biomass productivity (Pw), mortality rates (d), biomass (B), production rate (I de P) and final weight. Reproductive variables were: egg number/female (HPP), neonates number/female (NPP), egg maturity time (tm), first reproduction age (EPR), litter number (NC), reproduction frequency (FR), net reproduction rate (Ro) and generation time (Tc).

There were significant differences ($p<0.05$) on T2 from the population of adults, with concentrations of 15 ppm *S. cereviceae*, potato 15 ppm, soy oatmeal 30 ppm, with the highest specific growth, 0.50 ± 0.05 per day, less doubling time with 1.39 ± 0.14 days and the highest mortality rate with 0.49 ±0.07 per day. In the rest of the treatments there were no significant differences ($p>0.05$). There was evidence that the highest nutrient combinations strengthened the population growth in both adults and adults in juvenile reproductive stage. They reached in T6 with concentrations of 30 ppm *S. cereviceae*, potato 15 ppm, soy oatmeal 30 ppm, and 8.25 ± 1.70 and 15.0 ± 9.76 *Daphnias*/mL for each population respectively. Likewise in T6, was observed a higher value on egg number/female, 3.83 ± 0.82 and 3.55 ± 0.98, for each population respectively. *D. magna* presents a favorable adaptation under stress conditions, turning it into an excellent alternative for the living food for poslarvae production, with minimum infrastructure.

In order to use the *S. cereviseae* probiotic as an alternative of real control strategy, a meticulous evaluation must happen, evaluating their competing and functionality on the living food utilized with the different poslarvae species and their environments. It is imperative prerequisite to develop pathogenicity studies not only with the living food (*D. magna*) but also the selected probiotic from any commercial consideration; this means is necessary to explore this matter deeply (Austin & Brunt, 2009, as cited in Montet & Ray, 2009).

2. Cladocerans

Brachiopods are small crustacean with their legs flat as leafs. They can be found in freshwater habitats. Daphnia populations can be found in a range of water bodies, from huge lakes down to very small temporary pools, such as rock pools and vernal pools (seasonally flooded depressions). Often they are the dominant zooplankton and form, as such, an essential part of the food web in lakes and ponds. In many lakes, Daphnia are the predominant food for planktivorous fish, at least at times. As a consequence, the Daphnia species distribution and life history are closely linked with the occurrence of predators. Typically, Daphnia species are found in lakes with planktivorous fish they are smaller and more transparent than species found in fishless water bodies.

The cladocerans represent a key position in aquatic communities, not only as consumers herbivorous such as algae and bacteria but also as feedstuff for fish, birds and other aquatic predators (Dodson & Frey, 2001, as cited in Thorp & Covich, 2001; Brett et al., 2009, as cited in Arts et al., 2009). Taxonomically, the Branchiopods are grouped into six orders, 29 families, including the revisions suggested by Thorp & Covich (2001). Branchiopoda has the following orders and families. Order: Anomopoda, Ctenopoda, Onychopoda, and Family: Daphniidae, Moinidae, Bosminidae, Ilyocryptidae, Macrothricidae, Neothricidae, Acantholeberidae, Ophryoxidae, Chydoridae, Sididae, Holopediidae, Podonidae, Polyphemdida, Cercopagidae. Order: Haplopoda and Family: Leptodoridae. Order: Anostracai, and Family: Artemiidae, Branchinectidae, Branchipodidae, Chirocephalidae, Linderiellidae, Polyartemiidae, Streptocephalidae, Thamnocephalidaev. Order: Spinicaudat and Family: Cyclestheriidae, Cyzcidae, Leptestheriidae, Limnadiidae. Order: Laevicaudataand and Family: Lynceidae. Order: Notostraca and Family: Triopsidae (Dodson & Frey, 2001, as cited in Thorp &. Covich, 2001; Kobayashi et al., 2008, as cited in Suthers & Rissik, 2008).

2.1 Anatomy and physiology

The cladocerans do not have a segmented body, but they have a second segmented antenna. Most of them contain only one composed central eye during their adult stage and a clear transparent yellowish shell. Crustaceans are different from other arthropods because they have two pairs of the antennas. Cladocerans have a first pair of antennas (antennules, generally with one segment and other smaller antenna with chemical sense functions). The second pair of antennas is big and used to swim (Dodson &Frey, 2001, as cited in Thorp & Covich, 2001). Likewise other crustaceans, cladocerans mostly present in their heads: two pairs of antennas, one pair of jaws and two pair of jawbones. On the base of the head close to the shell they have a pair of short appendixes (antennules), normally this are shorter than the head and less visible, but sometimes can be longer (Moina) (Dodson &Frey, 2001, as cited in Thorp & Covich, 2001).

Behind their heads the bodies are composed by a thorax and an abdomen. The body finishes on a pair of claws (post-abdominal) that can show up from the shell. The chemistry of the shell is important, because cladocerans tend to have a hydrophobic exoskeleton, composed by chitin. The thorax and the abdomen displace in their shells when they are alive. The flat legs (called "phyllopod") have lines of mushrooms and spines that are used for feedstuff management, filtering, scraping and pumping out. The shell has a double wall, and between them there is a hemolymph flow, being part of the corporal cavity (Ebert, 2005).

The thoracic legs operate as electrostatic filters (not sifter), they collect algae and other particles that get attached to the flat surfaces and the mushroom combs. The intestine goes along with the mouth, in curves, continues over the body passing the thorax and the abdomen, and ends up in the anus close to the very end of the animal. It is divided in three regions. The previous intestine (water absorption using columnar cells) and the posterior intestine both aligned with the cuticle that wraps the exterior of the animal. The middle intestine (in the thorax) is aligned with the epithelium covered by microvillus, absorption site. On the head region of the Daphnia there is a pair of small bags (hepatic caecum) associated with the intestines. The heart is a muscular organ above the intestine and previous to the head. Cladocerans are between 0.5 mm and 6 mm long (Dodson & Frey, 2001, cited in Thorp &Covich, 2001; Ebert, 2005).

Males are distinguished from females by their smaller size, larger antennules, modified post-abdomen, and first legs, which are armed with a hook used in clasping. The genus Daphnia includes more than 100 known species of freshwater plankton organisms found around the world (figure 1) (Dodson & Frey, 2001, cited in Thorp &Covich, 2001).

3. Life cycle and development

Cladocerans have sexual and non-sexual reproduction, according to the environmental conditions; this is shown in figure 2. Most of the time the majority of the females has sexual reproduction (Dodson & Frey, 2001, cited in Thorp & Covich, 2001) and they develop eggs in a resting stage. Embryogenesis starts and ends in the incubation chamber, this is a space between the body and the shell. There are three types of eggs: - Diploid, they develop immediately in juveniles, - Resting eggs that come from haploid eggs, fertilized in embryo in early stages, the go on diapauses (resistant to heat, dryness and heating), - Pseudo-sexual eggs, this are diapausic embryos from non-sexual diploid eggs (Dodson & Frey, 2001, cited in Thorp & Covich, 2001; Ebert, 2005).

The non-sexual reproduction modality is an important characteristic in the implementation of controlled laboratory productions (by cyclical parthenogenesis). It is shown mainly that when there are satisfactory development conditions, producing female litters exclusively, that are able to succeed to the reproductive phase and to continue reproducing in a non-sexual way as long as the favorable conditions continue; feeding, low population density, and the main environmental factors and the water chemical quality maintain the adequate levels (Ebert, 2005).

Deteriorating environmental conditions stimulate the production of males; part of the brood will be established by males. When these males develop, they can give the possibility of the sexual reproduction fertilizing the females, and also a sexual resting eggs enclosed by anephippium, resistant structure, a resistant case made from the exoskeleton around the brood chamber. They contain one or two embryos (depending on the specie) on a sleeping stage, and they maintain the diapauses until the environmental conditions are suitable to restart the development of the embryo and then emerge juveniles that are able to start a new non-sexual reproductive cycle.

In the parthenogenesis reproductive system, the embryo development is direct and takes place on the female incubation chamber. From this chamber emerge juvenile organisms (neonates); they are freed to the environment when they complete its own development. The

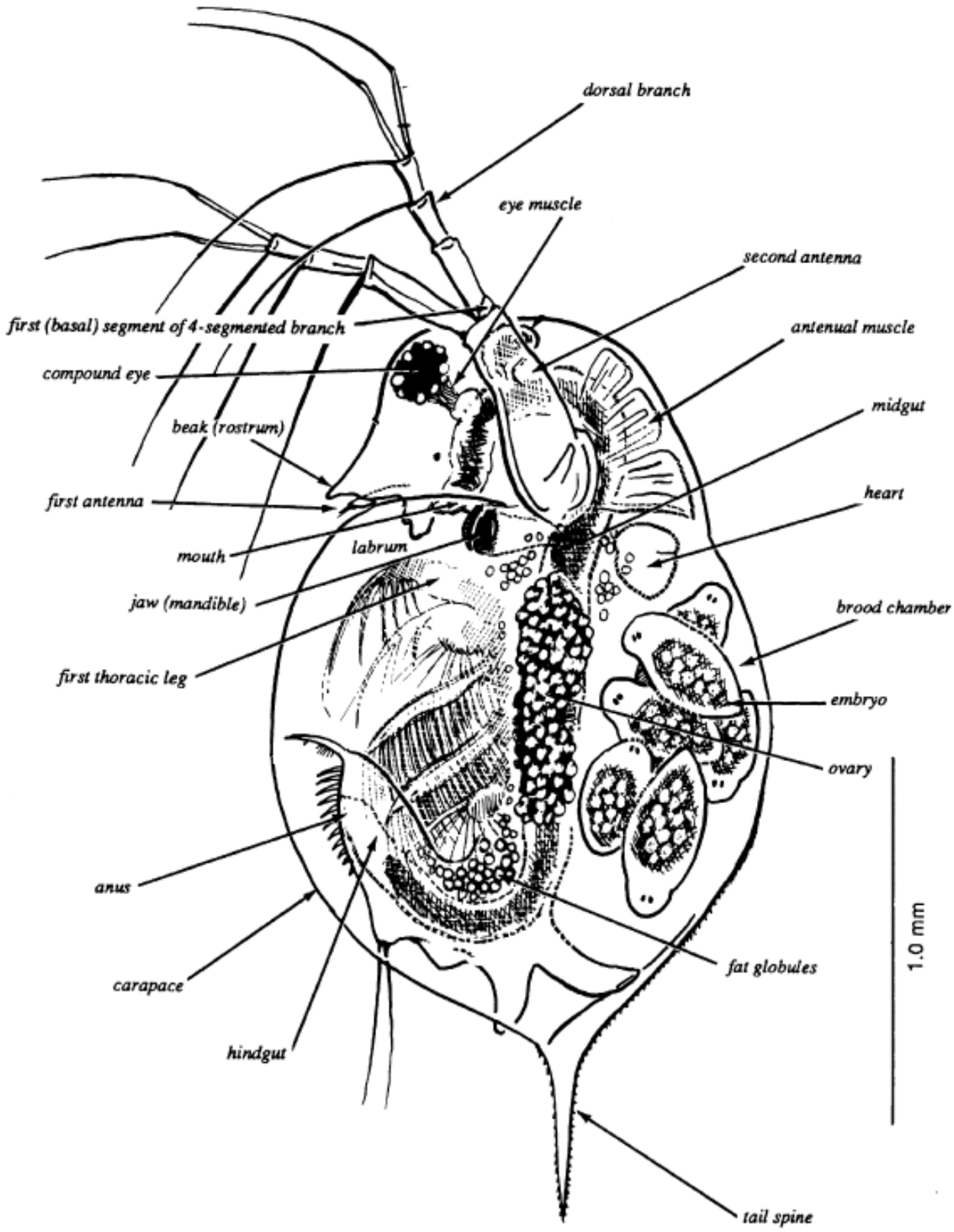


Fig. 1. Schematic drawing of the internal and external anatomy of retrieved *Daphnia*. Retrieve from (Lavens & Sorgeloos, 1996)

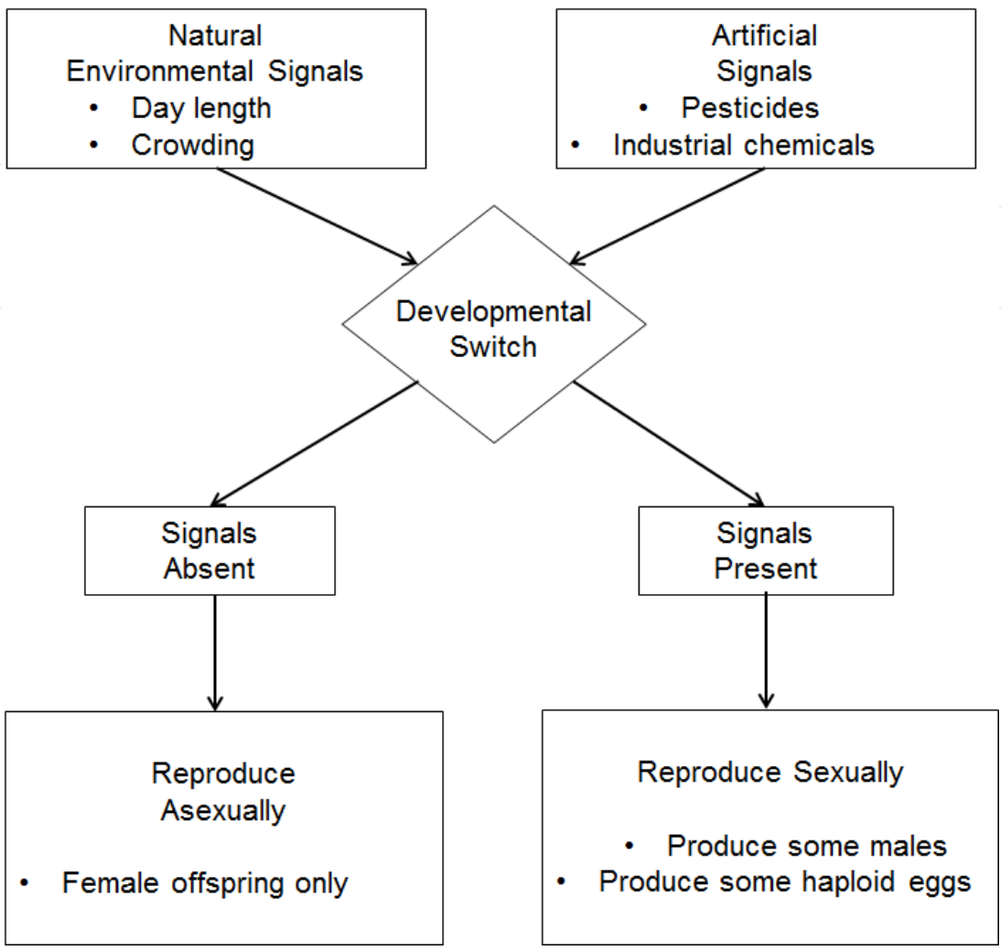


Fig. 2. Daphnia reproductive strategies. Female adults can reproduce on three different types of offspring, depending on the environmental conditions. Diploid eggs (emergent) reproduce non-sexually, develop in females and males. Haploid eggs are reproduced based on an exact chemical signal from the environment, Retrieve from (Thorp & Covich, 2001)

general appearance of the released juveniles is similar to the appearance of the adult, obviously with a minor size (Mitchell et al., 2004 as cited in American Public HealthAssociation [APHA], 2002).

4. Production of cladocerans

To keep a *Daphnia sp* production, it is required a basic laboratory infrastructure, expecting to have materials, equipment, reactive substances and organic strains such as algae and cladocerans. Daphnia has been used to evaluate chronicle and severe chemicals effects, and they are very sensitive to toxic substances. Breeding Daphnia allows to clone establishment with few genetic variation and reproductive results (American Public HealthAssociation, 2002).

4.1 Laboratory infrastructure

Laboratories should contain the minimal infrastructure necessary to develop controlled culture of micro-algae and to maintain cladocerans strains, which will be used for nutritional tests.

4.1.1 Materials

The containers used to grow the cladocerans reproducers should be made of the boron-silicate glass, adequate volume, depending on the production protocol that is used. They must be submitted to the appropriate cleaning routine and disinfection. To achieve these tests, enough and adequate glassware and minor equipment will be used to guarantee safe and repeatable proceedings, equipment like automatic calibrated pipettes, gagged glassware with the required capacity, stainless steel spatulas, etc.

4.1.2 Equipment

The essential equipment required is: analytical scale, optical microscope, stereomicroscope, autoclave (propane or electric), sterilized air or laminar flow hood for microbiological work, potentiometer, oximeter, salinometer, conductivimeter and spectrophotometer. It will be best to have a bioclimatic chamber for temperature control, lighting and photoperiods.

4.1.3 Reactives

Is necessary to have the indispensable reactives to prepare the dilution water (every time that cladoceran *Daphnia magna* production is made, it is essential to prepare the dilution water with the hardness specification required) (American Public Health Association, 2002), and culture medium, not only cladocerans but also for micro-algae. The compound should be analytical grade and free of contamination, as heavy metals. It is suggested the known commercial brands.

On each case it is suggested to prepare concentrated solutions (standard) and from this build up the culture medium and the dilution water. It must have a control over the proceedings to prepare the standard solutions and the culture medium and special attention on preserving substances that may be able to degrade or contaminate (vitamin solutions for example); in all cases it is necessary to apply the maintenance conditions to avoid the alteration on chemical concentration from the standard solutions.

5. Organisms for testing

Daphnia is widely distributed, this make it easy to obtain not only in laboratories but also in research institutions. 20 to 30 organisms are plenty to start the production. Additionally the reproduction of Daphnia allows establishing clones with low genetic variability and reproductive results (Martinez et al., 1998, as cited in Ramirez y Mendoza 2008). Some authors prefer other species of Daphnia, different from *Daphnia magna* because they have bigger neonates and some fish cannot use them for food (Prieto, 2001).

The Cladocerans species mentioned in this essay have been isolated from the Antioquia State (Ocampo et al., 2010). According to Martinez et al., (1998) the organisms used to do this evaluation must be obtained from laboratories with controlled strains. They can be obtained by purchasing them from distributors of the scientific material; this will guarantee

specific and good quality biological material. It is possible to obtain them using recollection although in this case they will have to develop the harvest to an F2, also they must acquire an identity certificate issued by an accredited institution, in this institution they will have to deposit a fixed or live sample, this way it will get a catalog or collection code. It is not allowed to use organisms purchased as feedstuff on a pet store, because the origin, history and quality is unknown. Frequently this material does not guarantee reproducibility and this make the results less reliable (Martinez et al., 1998, as cited in Ramirez y Mendoza 2008, as cited in Ramirez y Mendoza 2008).

5.1 Water

Daphnia can be maintained in reservoirs of natural water; however it is suggested an environment of synthetic water and should be standard quality reconstituted in order to generate predictive results and allow an adequate reproduction and growth of the culture. Hardness water recommended is 160-180 mg CaCO3/L, dissolved in deionized water and aerated for few hours. pH closed to 8.0. Normally stays between 7.0 and 8.6, no monitoring or adjustment necessary. Requirements to prepare the hard water: 192 mg/L of NaHCO3, 120 mg/L of CaSO-4, 120 mg/L mgSO4, and 8.0 mg/L of KCl (American Public Health Association, 2002).

5.2 Food and fed

Daphnias usually are fed *in vitro* with different type of algae, generally *Ankistrodesmus falcatus, Selenastrun capricornutum,* and *Clorella.* A mixture is prepared in sterilized water, adding two drops of solution per each Daphnia adult culture. Another diet to use is adding 6.3 g of trout pellet, 6.2 g of yeast and 0.5 g of alfalfa. Mix for 5 minutes with sterilized water, gauge to 500mL, decant for an hour and get rid of the surplus. Freeze 50 to 100 mL portions and do not save it for more than eight days. Use It to feed only 0.5 mL per each 1000 mL of culture medium, with a 3 times a week frequency; in an aerated environment and replaced every week (American Public Health Association, 2002). The yeast (*Saccharomyces cereviseae*) is a probiotic. FAO (2001) declare that probiotics are "living microorganisms, when managed in adequate amounts, present to the host health benefits" (Austin & Brunt, 2009, as cited in Montet & Ray, 2009).

5.3 Temperature

Temperature changes can induce death or induce ephippium production or sexual eggs. The ideal temperature is between 20°C and 25°C.

5.4 Light

Light intensity variations between 538 and 1076 lux and the prevalence of the light during the day/night cycles; do not affect the reproduction and growth of Daphnia cultures significantly. Try to provide a minimum of 12 hours of light / 12 hours of darkness.

5.5 Containers

Glass or plastic containers should be used, in order to observe easily the cultures, they should have a capacity of 3 L, with 2.75 L of medium, and 30 Daphnias, will be able to

produce approximately 300 neonates and young Daphnias per week. It is necessary to clean weekly, removing the food left and the death Daphnias. Wash the containers monthly with detergent and replace the medium completely.

5.6 Air supply

Daphnia can survive with oxygen levels up to 3 mg/L, but to grow requires 6 mg/L. To aerate the harvest, it is possible to use motors to aerate aquariums.

5.7 Maintenance of cultures

The media should be replaced weekly; it is not necessary if massive media (100 L) is used. Approximately 30 Daphnias per culture should be selected every week; to retrieve them a glass or plastic Pasteur pipette must be used. This activity is suggested to do at the same time when the medium is changed, this system will avoid cross-linking (American Public Health Association, 2002).

5.8 Selection of organisms for test

The examination should initiate with the second or third offspring, selecting neonates with ≤ 24 birth hours. Before the initiation any test, 30 to 40 young Daphnias should be isolated on a 400 mL beaker with 300 mL of algae mix (*Ankistrodesmus falcatus, Selenastrun capricornutum,* and *Clorella*). Do not use ephippiums for Daphnia cultures, because its presence is an indicator of unfavorable conditions (American Public Health Association, 2002).

6. Methodology

Potatoes (*Solanum tuberosum L)* and yeast (*Saccharomyces cereviceae)* contain more than 60% of protein, this consumption by the post- larva is consistent with the protein requirement, and also this source contains immune system stimulant compounds such as β-glucan, nucleic acids like manna oligosaccharides that increase the immune response (Champagne et al., 2009). When yeast gets attached to the intestine of the larvae, on their 27th day after birth, it produces a higher secretion of amylase and this stimulates the enzymes located on the membrane cells in the brush border shape (Austin & Brunt, 2009, as cited in Montet & Ray, 2009).

Potatoes (*Solanum tuberosum L*) have been used as a nutrient because they play an energetic roll due to their high levels of starch (60% – 80%), of the dry matter is starch. In addition, the potato is low in fat and rich in several micronutrients, especially vitamin C. It is also a good source of vitamins B1, B3, B6, folate, pantothenic acid, riboflavin and minerals, such as potassium, phosphorus and magnesium, Potatoes also contain dietary antioxidants, which may play a part in preventing diseases related to ageing (Murniece et al., 2011; FAO, 2008a).

The enrichment with flour provides protein (50%) and high level of lecithin to the living food. Few authors have shown the incidence in growth and intestine morphology improvement including soy oatmeal (Murray et al., 2010). Despite its limitations, the incidence of scoliosis and mouth twisting in the larvae is reduced with the addition of lecithin Salze et al., 2010). Gutiérrez-Espinoza and Vásquez-Torres (2008), confirm that soy

in different presentations has a high digestibility index in White Cachama juveniles (*Piaractus brachypomus*) and can be used without restrictions on this specie (Guillaume et al., 2004; Sealey et al., 2009).

The mutual action of these three nutrients over the productive and reproductive parameters of Daphnia magna can be demonstrated with this research, thorough its exposition to eight diets. These diets include yeast, potato and soy oatmeal, with 15 and 30 ppm concentrations of nutrient and enrichment compound, determining productive and reproductive parameters, creation of life history and comparing populations under stress conditions.

Daphnia magna was used, with two populations: one with adults and the other with young breeders (32 specimens / population). *Daphnia magna* was seeded individually (1 Daphnia/tank) in multicells with 24 tanks, each one with 3 mL capacity, covered with parafilm. The compartments were fully filled with the culture medium according to the treatment. The daily exchange for a culture medium was 50%. Concentrations of 30 mg/L and 15 mg/L were prepared for the culture medium for each of the nutrient with discolored water. The treatments used were the following:

Treatment 1: 15 ppm *S. cereviceae*, 15 ppm potato, 15 ppm soy oatmeal flour.

Treatment 2: 15 ppm *S. cereviceae*, 15 ppm potato, 30 ppm soy oatmeal flour.

Treatment 3: 15 ppm *S. cereviceae*, 30 ppm potato, 15 ppm soy oatmeal flour.

Treatment 4: 15 ppm *S. cereviceae*, 30 ppm potato, 30 ppm soy oatmeal flour.

Treatment 5: 30 ppm *S. cereviceae*, 15 ppm potato, 15 ppm soy oatmeal flour.

Treatment 6: 30 ppm *S. cereviceae*, 15 ppm potato, 30 ppm soy oatmeal flour.

Treatment 7: 30 ppm *S. cereviceae*, 30 ppm potato, 15 ppm soy oatmeal flour.

Treatment 8: 30 ppm *S. cereviceae*, 30 ppm potato, 30 ppm soy oatmeal flour.

The whole production was preserved in a laboratory with the following environmental conditions: 1.200 masl (meters above the sea level), 22 ± 3 °C room temperature, 12 light hours, 12 dark hours, 7.0 ± 0.6 pH water, during 20 days. Eight treatments were used with four replica/treatment.

These are the productive and reproductive parameters of Daphnia magna:

6.1 Growth and corporal weight: Productive variables

Maximum density (Dmax):

$$Dmáx = \frac{\text{Final population}}{\text{Volume}} \equiv \left[\frac{\text{Individuals}}{\text{Volume}}\right] \quad (1)$$

Daily average density (Dmd):

$$Dmd = \frac{\text{Final population}}{T} \equiv \left[\frac{\text{Individuals}}{\text{Time}}\right] \quad (2)$$

Doubling Time (Td):

$$Td=\frac{0.693}{k}\equiv [días] \tag{3}$$

Specific growth rate (k):

$$k=\frac{Ln(\text{ final population})-Ln(\text{PoblInitial population inicial})}{\text{TiempoTime}}\equiv \left[\frac{1}{day}\right] \tag{4}$$

Performance (r):

$$r=\frac{(\text{Final populationl})-(\text{Initial population})}{Time}\equiv \left[individuals*\frac{1}{Time}\right] \tag{5}$$

Numeric growth (PN):

$$P_N=b*\bar{N}\equiv \left[\#\ individuals*\frac{1}{Time}\right] \tag{6}$$

$\bar{N}$= individual media number
b= birth rate population, in a period of time.

Birth rate (b) (Edmodson equation):

$$b=\ln(E/D+1)\equiv[\#\ neonates] \tag{7}$$

E= egg number /female
D= egg development time (days).

Another way to calculate the birth rate is:

$$k=b+d \tag{8}$$

therefore:

$$b=k-d \tag{9}$$

Individuals average number ($\bar{N}$):

$$\bar{N}=\frac{(No+N_t)}{2} \tag{10}$$

No=individuals initial number
Nt=individual number after a period of time t

Biomass productivity (Pw):

$$P_W=b*\bar{N}*\bar{W}\equiv \left[\frac{Biomass}{Volume}*\frac{1}{Time}\right] \tag{11}$$

$\bar{W}$=Final weight at the end of the period.

$\bar{N}$= Individuals media number

b= birth rate population, in a period of time.

Mortality rate (d):

$$d=\frac{\text{Ln(Final pop. of neonates)-Ln(Initial pop. of neonates)}}{T}\equiv\left[\frac{1}{\text{day}}\right] \quad (12)$$

Biomass (B):

$$B=\frac{\text{(Average number)*(Final weigth)}}{T}\equiv\left[\frac{\text{Biomass}}{\text{Volume}}\right] \quad (13)$$

Production rate (I de P):

$$\text{I de P}=\frac{P}{B} \quad (14)$$

Final weight (pf) was determined as dry weight. Every Daphnia was dried up with tissue paper (analytical scale, 0.0001g. precision), expressed in mg/L.

6.2 Reproductive variables

Egg number/female (HPP). The egg number/female was determined in the microscope (10x and 40x).

Neonate number/female (NPP). This count was made directly.

The measurement of the rest of the variables was taken directly from the data base, such as:

Egg development time (tm), number of days passed between the appearance of the egg in the incubation chamber and the presence of the neonate.

Offspring number (NC)

First reproduction age (EPR), in days.

Production frecuency (FR), in hours.

The born individuals were moved to another tank with the same treatment. Neonate mortality was evaluated here.

6.3 Life history parameters

Daily measurements were made in 32 young breeders and in 32 adults, keeping a follow up during the 21 days (duration of the research). The frequency of the observation was 12 hours to differentiate seeded population and produced population.

The reproduction net rate (Ro) and the generation time (Tc), were calculated according to the Lotta equation (1913).

$$\sum_{X=0}^{n} l_x m_x (\exp^{-rx}) = 1 \tag{15}$$

lx= survival in a specific time starting in the birth period.
mx= fertility in a specific period
x=period (from 0 to 21 days)
r= intrinsic grow rate

Net reproduction rate (Ro):

$$R_o = \sum_{X=0}^{n} l_x m_x \tag{16}$$

Generation time (Tc):

$$T = {}^{1}/_{R_o} \sum_{X=0}^{n} l_x m_x X \tag{17}$$

Where Ro= net reproductive rate and Tc= generation time.

6.4 Statistical methodology

An experimental classification design was used, fully randomized, fixed effect, symmetric, balanced, 23 factorial arrangement, with 4 replicas, n=32 to each population. The results were compared through ANOVA and Tukey test with α=0.05. An exploratory descriptive analysis, one-dimensional for each condition type, was established. Data transformation to the square root function was used on egg number/female and neonates number variables. For survival and mortality percentages, arcsine transformation was applied. SAS version 9.1 Statistical package was used.

$$Y_{ijsk} = \mu + L_i + P_j + E_s + LP_{ij} + LE_{is} + PE_{js} + LPE_{ijs} + \varepsilon_{k(ijs)} \tag{18}$$

Where Y_{ijsk} : represented de number variable of *Daphnia magna.*

μ : Experiment average effect

L_i : Yeast effect

P_j : Potato dosage effect

E_s : Enrichment effect

$\varepsilon_{k(ijs)}$: Experimental error

Additionally, a comparison test with adult population and young breeders was made. To do this, the Mann Whitney test was applied, for the productive variables: daily average density (Dmd), doubling time (Td), specific growth rate (k), performance (r) and biomass productivity (Pw) and for reproductive parameters: egg number/female (HPP), neonates number/female (NPP), litter number (NC), net reproduction rate (Ro) and generation time (Tc). A correlation analysis was completed between the variables. For reproduction net rate and generation time, one way analysis of the variance and the Tukey test took place, in order to compare the life history parameters between the different diets.

7. Results

In the adult population were found significant differences ($p<0.05$) on the average treatment in the daily growth rate, duplication time and mortality. The daily growth rate from T1 to T8 presented the following values: 0.15 ± 0.05^{b}, 0.50 ± 0.05^{a}, $0.33 \pm 0.12^{a,b}$, $0.23 \pm 0.03^{a,b}$, $0.31 \pm 0.08^{a,b}$, $0.33 \pm 0.18^{a,b}$, $0.40 \pm 0.20^{a,b}$ and 0.19 ± 0.11^{a} cladocerans per day, respectively. The major growth rate occurred on T2 with 0.50 ± 0.05^{a} cladocerans per day, presenting a significant differences p (<0.05) in the treatment T1, that showed a value of 0.15 ± 0.05^{b} cladocerans per day compared with T2 with values of 0.50 ± 0.05^{a} cladocerans per day, as can be seen on table 1.

The duplication time of T1 to T8 had values of 4.96 ± 1.52^{a}, 1.39 ± 0.14^{b}, $2.31 \pm 0.90^{a,b}$, $2.99 \pm 0.48^{a,b}$, $2.27 \pm 0.53^{a,b}$, $2.80 \pm 1.83^{a,b}$, $2.10 \pm 1.06^{a,b}$and $4.40 \pm 2.43^{a,b}$ days respectively. This parameter presented significant differences p (<0.05) during the treatment T1, where a duplication time of 4.96 ± 1.52^{a} was observed, in relation to T2 that presented a minor duplication time with 1.39 ± 0.14^{b} days, as can be seen on table 1.

The mortality rate from T1 to T8 presented the following values: 0.15 ± 0.05^{b}, 0.49 ± 0.0^{a}, $0.32 \pm 0.12^{a,b}$, $0.21 \pm 0.04^{a,b}$, $0.31 \pm 0.08^{a,b}$, $0.31 \pm 0.17^{a,b}$, $0.40 \pm 0.20^{a,b}$, $0.21 \pm 0.10^{a,b}$, respectively. The highest mortality rate was presented in T2 with 0.49 ± 0.07^{a} cladocerans per day, presenting significant difference p (<0.05) betweenT1 with 0.15 ± 0.05^{b}cladocerans per day with regard to T2 with 0.49 ± 0.07^{a} cladocerans per day (table 2).

Even though there were no significant differences ($p>0.05$) between the treatment media in the rest of the evaluated parameters, not only in the adult population group, but also the young breeding group, it is important to notice in a general matter, that it was a better population performance in T2 and T6 (tables, 3, 4, 5 and 6). In T2 where growth rate was the highest with 0.37 ± 0.13^{a} cladocerans per day, the minor duplication time with 2.02 ± 0.59^{a} days, and the mayor performance with 1.23 ± 0.46^{a} cladocerans per period. Likewise T6 presented the highest maximum density and the mayor egg number per female with 15.0 ± 9.76^{a} and 8.25 ± 1.70^{a} cladocerans, and 5.0 ± 0.0^{a} and 3.83 ± 0.82^{a} eggs per female, respectively. This information can be seen on table 4.

7.1 Population comparisons

The daily average density (Dmd) of *D. magna* in the adult population was 4.0 individuals per cell and in the population of young breeders were 2.0 individuals. The maximum Doubling Time (Td) in adults was 2.43 days and in the population of young breeders was 12 days. The maximum Performance (r) was, in adults 2.7% and in young breeders 0.58%. The

Treatments	Adult Populations					Young Breeder populations				
	Dmd (Clad/vol.) x ± DE	Dmáx (Clad/vol.*d) x ± DE	k (Clad/day) x ± DE	Td (days) x ± DE	r (Clad/vol.*d) x ± DE	Dmd (Clad/vol.) x ± DE	Dmáx (Cla/vol*d) x ± DE	k (clad/day) x ± DE	Td (days) x ± DE	r (Clad/vol.*d) x ± DE
1	4.7±4.78a	0.42±0.47a	0.18±0.08 a	4.34±2.05 a	0.66±0.47 a	4.0 ±2.94 a	0.48 ±0.31a	0.15±0.05 b	4.96±1.52 a	0.35±0.27 a
2	6.25±3.86a	1.12±0.72a	0.37±0.13 a	2.02±0.59 a	1.23±0.46 a	4.5±4.35 a	1.66±0.57 a	0.50±0.05 a	1.39±0.14 b	1.56±0.33 a
3	2.75±3.5 a	0.29±0.15 a	0.14±0.0 a	5.0±0.0 a	0.47±0.0 a	10.75 ±3.2 a	1.86 ±0.75 a	0.33±0.12 a, b	2.31±0.90 a, b	1.42 ±0.70 a
4	4.25±2.75 a	0.47±0.40 a	0.16±0.13 a	7.65 ±7.32 a	0.43±0.38 a	8.0±4.58 a	0.98±0.20 a	0.23±0.03 a, b	2.99 ±0.48 a, b	0.71 ±0.32 a
5	4.75±4.92a	0.60±0.63 a	0.20±0.12 a	5.1±4.29 a	0.63±0.65 a	10.25±2.69 a	1.47±0.59 a	0.31±0.08 a, b	2.27 ±0.53 a, b	1.26 ±0.61 a
6	8.25±1.70 a	0.82±0.45 a	0.22±0.15 a	3.94±1.61 a	0.71±0.36 a	15.0±9.76 a	1.91±1.48 a	0.33±0.18 a, b	2.80±1.83 a, b	1.76 ±1.46 a
7	7.75±5.37 a	0.67±0.38 a	0.20±0.08 a	3.66±1.19 a	0.75±0.10 a	9.75±7.22 a	1.84±0.91 a	0.40±0.20 a, b	2.10 ±1.06 a, b	1.39 ±0.99 a
8	7.75±2.87 a	0.50±0.18 a	0.12 ±0.02 a	5.72±1.31 a	0.41±0.19 a	10.66±7.50 a	1.07±0.82 a	0.19±0.11 a, b	4.40 ±2.43 a, b	0.91 ±0.89 a

Dmáx: maximum density; Dmd: Daily media density; k: intrinsic growth rate; Td: Duplicating time; r: Performance. DE= Standard deviation. Different letters indicate statistical difference ($p<0.05$) between columns, per population.

Table 1. Average ± DE, for population parameters during the evaluated period in two populations of *Daphnia magna,* according to treatment

Treatments	Adult Populations					Young Breeder populations				
	Pw (mg/mL*day) x ± DE	B (mg/mL) x ± DE	P/B (I de Prod./day) x ± DE	b (Clad/day) x ± DE	d (Clad/day) x ± DE	Pw (mg/mL*day) x ± DE	B (mg/mL) x ± DE	P/B (I of Prod/day) x ± DE	b (Clad/day) x ± DE	d (clad/day) x ± DE
1	.	.	.	0.57± 0.27 [a]	0.39 ± 0.19 [a]	1.25 ± 1.36 [a]	3.5 ± 2.18 [a]	0.30 ± 0.15 [b]	0.34 ± 0.15 [a]	0.15 ± 0.05 [b]
2	3.06±0.0 [a]	5.9 ± 0.0 [a]	0.52 ± 0.0 [a]	0.64 ± 0.10 [a]	0.27 ± 0.13 [a]	2.55 ±1.17 [a]	5.22 ± 2.21 [a]	0.48± 0.02 [a]	0.48± 0.02 [a]	0.49±0.07 [a]
3	2.07±0.0 [a]	7.47±0.0 [a]	0.28 ± 0.0 [a]	0.28 ± 0.0 [a]	0.14 ± 0.0 [a]	2.81 ± 0.0 [a]	5.32 ± 0.0 [a]	0.53 ± 0.00 [a]	0.38 ± 0.24 [a]	0.32 ± 0.12 [a,b]
4	6.48±0.0 [a]	7.3 ± 0.0 [a]	0.89 ± 0.0 [a]	0.53 ± 0.34 [a]	0.37 ± 0.21 [a]	4.49 ± 3.71 [a]	7.87 ± 6.02 [a]	0.54 ± 0.07 [a]	0.58 ± 0.06 [a]	0.21 ± 0.04 [a,b]
5	12.15± .0 [a]	12.8 ± 0.0 [a]	0.95 ± 0.0 [a]	0.55 ± 0.35 [a]	0.35 ± 0.28 [a]	4.07 ± 2.05 [a]	7.69 ± 3.58 [a]	0.53 ± 0.04 [a]	0.54 ± 0.04 [a]	0.31 ± 0.08 [a,b]
6	5.44±0.77 [a]	7.15 ± 1.08 [a]	0.77 ± 0.17 [a]	0.74 ± 0.16 [a]	0.52 ± 0.03 [a]	7.63 ± 8.69 [a]	12.18 ± 8.30 [a]	0.51 ± 0.23 [a]	0.52 ± 0.22 [a]	0.31 ± 0.17 [a,b]
7	7.68 ± 6.59 [a]	9.31 ± 5.52 [a]	0.74 ± 0.26 [a]	0.65 ± 0.24 [a]	0.44 ± 0.27 [a]	5.29 ± 4.69 [a]	8.14± 6.15 [a]	0.56 ± 0.19 [a]	0.57 ± 0.18 [a]	0.40 ± 0.20 [a,b]
8	6.27 ± 4.61 [a]	9.35 ± 3.52 [a]	0.58 ± 0.28 [a]	0.58 ± 0.28 [a]	0.45 ± 0.26 [a]	5.48 ± 6.03 [a]	8.95 ± 6.75 [a]	0.45 ± 0.32 [a]	0.48 ± 0.30 [a]	0.21 ± 0.10 [a,b]

Pw: Biomass productivity; B: Biomass; PB: Productivity index, b: birth rate and d: Mortality rate. DE= Standard deviation. Different letters indicate statistical difference ($p<0.05$) between columns, per population.

Table 2. Average ± DE, for population parameters during the evaluated period in two populations of *Daphnia magna,* according to treatment

Treatments	Adult Populations				Young Breeder Populations			
	NPP (Neona/Fem.) x ± DE	NCAM Birth/perd. x ± DE	EPR (days) x ± DE	FR (hours) x ± DE	NPP (Neona/fem.) x ± DE	NCAM birth/perd. x ± DE	EPR (days) x ± DE	FR (hours) x ± DE
1	7.50 ± 3.53 [a]	2.50 ± 0.70 [a]	13.0 ± 2.82 a	48.0 ±33.94 [a]	4.0 ± 2.64 [a]	1.33 ± 0.57 [a]	5.0 ± 0.0 [a]	7.50 ± 3.53 [a]
2	7.0 ± 2.0 [a]	2.0 ± 0.0 [a]	14.33 ± 0.57 [a]	76.0 ± 18.33 [a]	7.0 ± 2.82 [a]	1.50 ± 1.73 [a]	2.5 ± 2.12 [a]	7.0 ± 2.0 [a]
3	7.0 ± 0.0 [a]	3.0 ± 0.0 [a]	13.0 ± 00 [a]	60.0 ± 00 [a]	9.75 ± 3.20 [a]	4.25 ±1.25 [a]	2.75 ± 0.50 [a]	7.0 ± 0.0 [a]
4	4.33 ± 2.08 [a]	1.66 ± 0.57 [a]	14.0 ± 1.0 [a]	42.0 ± 8.48 [a]	8.50 ± 4.79 [a]	2.75 ± 1.25 [a]	4.0 ± 2.58 [a]	4.33 ± 2.08 [a]
5	5.0 ± 5.19 [a]	2.0 ± 1.0 [a]	13.0 ± 1.0 [a]	84.0 ± 31.17 [a]	9.25 ± 2.62 [a]	2.5 ± 0.57 [a]	4.75 ± 2.62 [a]	5.0 ± 5.19 [a]
6	7.25 ± 1.70 [a]	3.0 ± 0.81 [a]	13.75 ± 1.5 [a]	45.0 ± 24.7 [a]	14.0 ± 9.76 [a]	3.50 ± 2.08 [a]	4.33 ± 2.51 a	7.25 ± 1.70 [a]
7	9.0 ± 3.60 [a]	2.66 ± 0.57 [a]	12.0 ± 1.0 [a]	92.0 ± 45.4 [a]	8.75 ± 7.22 [a]	2.25 ±1.50 [a]	3.50 ± 1.00 a	9.0 ± 3.60 [a]
8	6.75 ± 2.87 [a]	2.0 ± 0.81 [a]	14.25 ± 1.28 [a]	36.0 ± 12.0 [a]	11.0 ± 6.68 [a]	2.75 ± 1.25 [a]	4.33 ± 2.88 a	6.75 ± 2.87 [a]

NPP: Neonates per female, NCP: Offspring number, EPR: First reproduction age y FR: Reproduction frequency. DE= Standard deviation. Different letters indicate statistical difference ($p<0.05$) between columns, per population.

Table 3. Average ± DE, for population parameters during the evaluated period in two populations of *Daphnia magna,* according to treatment

Treatments	Female Adult Population		Young Breeder Population	
	HPP (N/female/day) x ± DE	D (days) x ± DE	HPP (N/female/day) x ± DE	D (days) x ± DE
1	2.36 ± 0.89 [a]	3.75 ± 1.0 [a]	1.8 ± 0.50 [a]	5.0±2.82 [a]
2	2.26 ± 1.57 [a]	3.33 ± 0.28 [a]	1.79 ± 0.34 [a]	2.5±0.70 [a]
3	1.72 ± 0.32 [a]	4.33 ± 0.0 [a]	2.24 ± 1.04 [a]	2.85 ± 1.03 [a]
4	2.53 ± 0.61 [a]	4.33 ± 2.51 [a]	3.46 ± 1.07 [a]	2.77 ± 1.18 [a]
5	2.7 ± 1.0 [a]	4.39 ± 1.66 [a]	3.05 ± 0.97 [a]	3.41 ± 0.68 [a]
6	3.83 ± 0.82 [a]	3.54 ± 0.76 [a]	3.55 ± 0.98 [a]	3.36 ± 2.76 [a]
7	3.23 ± 1.08 [a]	4.16 ± 1.25[a]	2.81 ± 1.19 [a]	2.69 ± 1.33 [a]
8	3.63 ± 1.17 [a]	5.20 ± 2.07 [a]	3.19 ± 1.60 [a]	2.49 ± 1.37 [a]

HPP: Egg number, D: Development time. DE= Standard deviation. Different letters indicate statistical difference ($p<0.05$) between columns, per population.

Table 4. Average ± DE, for population parameters during the evaluated period in two populations of *Daphnia magna,* according to treatment

Treatments	Female Adult Population			Young Breeder Population		
	Pf (mg/mL) x ± DE	PN (Ind*mL) x ± DE	N average (Cladoc.) x ± DE	Pf (mg/mL) x ± DE	PN (Ind*mL) x ± DE	N average (Cladoc.) x ± DE
1	1.77± 0.26 [a]	0.70 ± 0.70 [a]	2.0 ± 1.32 [a]	2.03 ± 0.0 [a]	2.67 ± 2.17 [a]	3.0 ± 2.5 [a]
2	1.57 ± 0.30 [a]	3.59 ± 1.28 [a]	3.75 ± 1.76 [a]	1.97± 0.0 [a]	2.64 ± 1.03 [a]	4.0 ± 1.0 [a]
3	1.28 ± 0.49 [a]	3.75 ± 2.03 [a]	5.37 ± 1.60 [a]	2.05 ± 0.25 [a]	1.11 ± 0.0 [a]	4.0 ± 0.0 [a]
4	1.70 ± 0.42 [a]	1.76 ± 0.96 [a]	4.37 ± 2.52 [a]	2.03 ± 0.56 [a]	1.46 ± 1.22 [a]	2.33 ± 1.15 [a]
5	1.90± 0.48 [a]	3.22 ± 1.67 [a]	4.87 ± 1.25 [a]	2.01 ± 0.16 [a]	2.24 ± 2.98 [a]	3.0 ± 2.59 [a]
6	1.69 ± 0.21 [a]	5.13 ± 5.42 [a]	7.37± 4.85 [a]	1.89 ± 0.23 [a]	2.96 ± 0.17 [a]	4.12 ± 0.85 [a]
7	1.91 ± 0.14 [a]	3.49 ± 2.98 [a]	4.50 ± 3.67 [a]	1.98 ± 0.16 [a]	3.44 ± 2.31[a]	5.0 ± 1.80 [a]
8	1.78 ± 0.08 [a]	3.16 ± 2.57 [a]	5.87 ± 3.56 [a]	2.66 ± 0.49 [a]	2.35 ± 1.82 [a]	3.62 ± 1.43 [a]

Pf: Final weight, PN: Numeric average, N prom: Number average. DE= Standard deviation. Different letters indicate statistical difference ($p<0.05$) between columns, per population.

Table 5. Average ± DE, for population parameters during the evaluated period in two populations of *Daphnia magna,* according to treatment

Treatments	Female Adult Population		Young Breeder Population	
	Reproductive net rate 1/day x ± DE	Regenerating time Days x ± DE	Reproductive net rate 1/day x ± DE	Regenerating time Days x ± DE
1	6.33 ± 0.00 [a]	7.00 ± 0.00 [a]	7.5±3.53[a]	15.75 ± 7.42 [a]
2	7.00 ± 2.82 [a]	8.00 ± 2.82 [a]	6.74 ± 2.08 [a]	16.48 ± 4.67 [a]
3	9.63 ± 3.21 [a]	11.00 ± 3.46 [a]	7.00 ± 0.0 [a]	15.00 ± 0.00 [a]
4	8.50 ± 4.79 [a]	13.50 ± 0.70 [a]	4.33 ± 2.08 [a]	14.46 ± 6.31 [a]
5	9.08 ± 2.32 [a]	9.00 ± 1.00 [a]	5.16 ± 5.05 [a]	13.93 ± 1.88 [a]
6	13.55 ± 9.30 [a]	14.00 ± 11.26 [a]	7.5 ± 1.91 [a]	15.56 ± 5.13 [a]
7	10.00 ± 7.50 [a]	10.33 ± 8.62 [a]	8.65 ± 4.40 [a]	6.06 ± 10.47 [a]
8	11.18 ± 6.65 [a]	11.75 ± 7.58 [a]	6.95 ± 2.53 [a]	12.08 ± 7.48 [a]

Ro: Reproductive net rate; Tc: Regenerating time. DE= Standard deviation. Different letters indicate statistical difference ($p<0.05$) between columns, per population

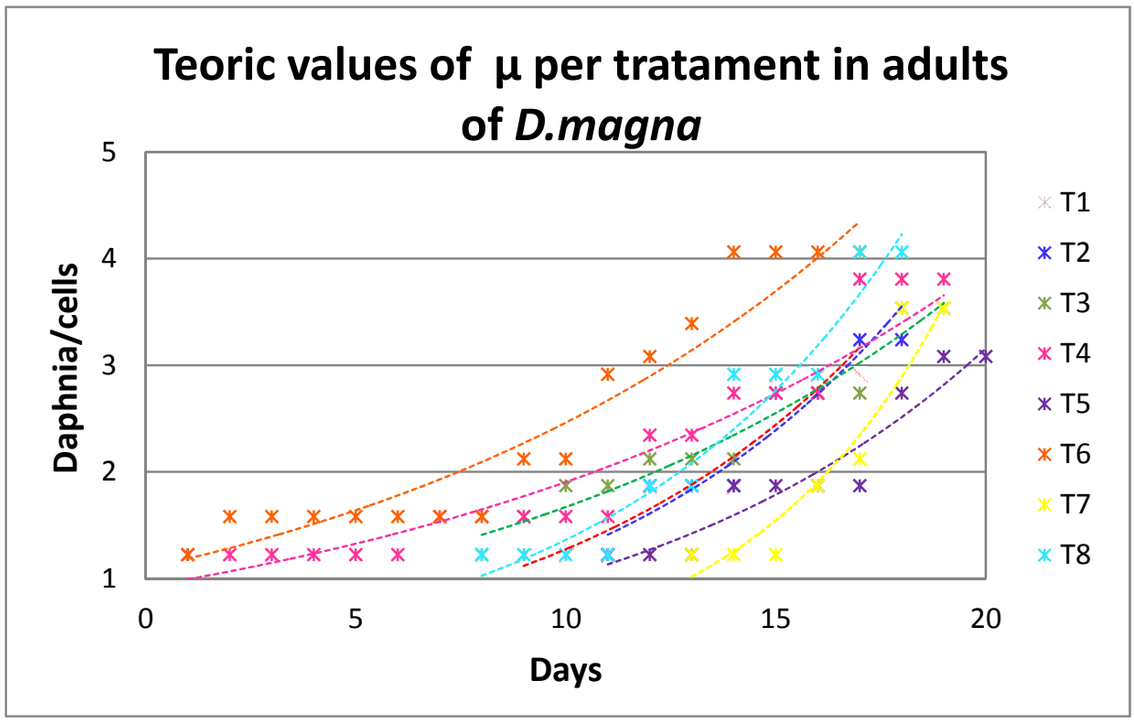
Table 6. Reproductive net rate, regenerating time in two populations of *Daphnia magna,* according to treatment

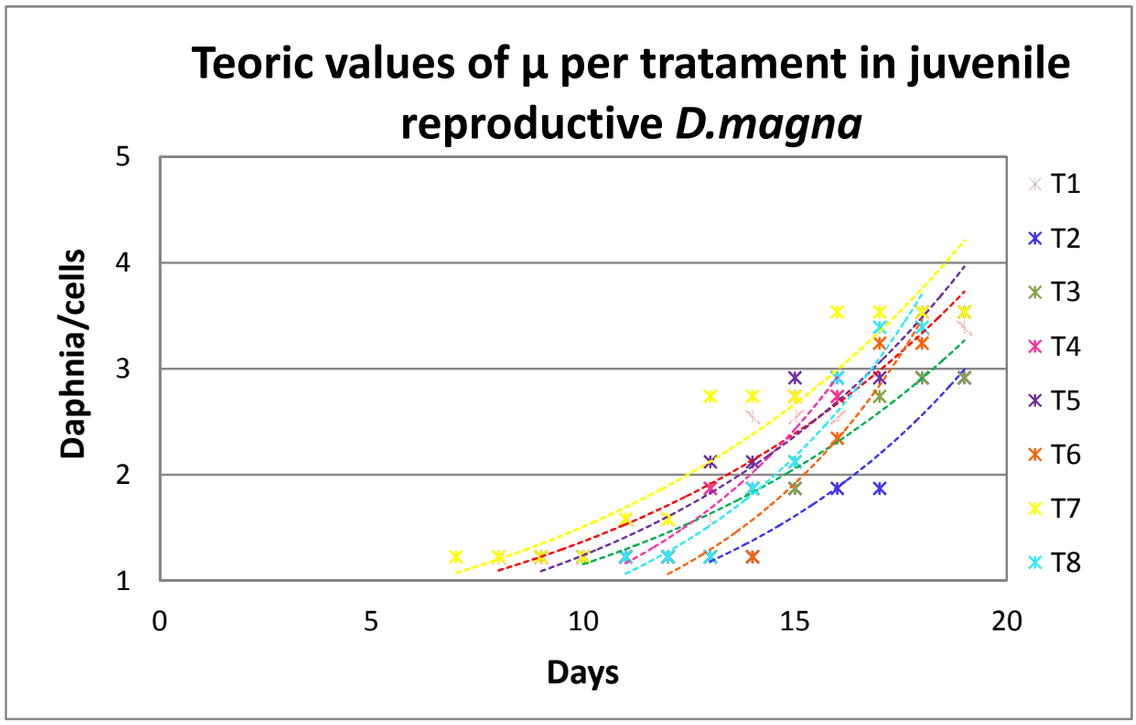
Specific growth rate (k) was 0.65 cladocerans per day in adults and 0.52 in young breeders. The maximum Biomass (B) was 24.73 mg/cell in adults and 13.22 mg/cell in the population of young breeders.

Adult *D.magna* presented a maximum value of 27 neonates/ adult female per each volume unit during the evaluation period, different from the population of young breeders when their fecundity levels reached 12 neonates/female.

The offspring maximum number was 6 offspring/period for the adult population, and 4 offspring/period for the population of young breeders. The maximum age for the first reproduction was 7 days in adults, meanwhile in the population of young breeders was 16 days. The maximum level of reproduction frequency in the adult population was 216 hours and for the population of young breeders was 120 hours.

In the following figures 3 and 4, a μ adjustment or specific growth rate founded experimentally can be observed. The regression coefficient for the treatments on the adult population oscillated between r (0.8966 – 0.9364). The dots in both graphics correspond to the experimental data and the curves correspond to de adjustments. In the graphics the differences in the growth rate for both populations can be observed as well. Note that the adult population it is found in a reproductive process and growth in the beginning of the experiment, meanwhile the population of neonates must conquer its maturity to initiate reproduction and growth.





Figs. 3. and 4. Population growth of *D. magna*. The trajectory shows an exponential growth with μ values common for cladocerans. Correspond to nutritional situation, specific conditions of each treatment, depreciable mortality and water temperature closed to 20°C.

7.2 Interactions

Even though there was not statistic relation ($p>0.05$) between the parameters of maximum density, daily media density and final weight on the adult population, there was a nutrient significant correlation ($p<0.05$): The yeast nutrient was highly significant ($p<0.01$) on the 30 ppm concentration, on the maximum density with 11.46 ± 6.79 cladocerans per volume, while the treatments with a concentration of 15 ppm of yeast, had 6.7 ±4.43 cladocerans per volume, on a concentration of 30 ppm. Similarly happened with the final weight, that presented values of 1.63 ± 0.35 mg in 15 ppm and 1.82 ± 0.26 in 30 ppm, presenting a mayor value on final weight on the diet with 30 ppm yeast concentration.

A highly significant relation was found (<0.01) between the three nutrients. With yeast and potato, with concentrations of 30 ppm and 15 ppm respectively, the highest value on the variable maximum density was found, with 12.62 ± 7.0 cladocerans per volume. The daily media density presented significance ($p<0.05$) in the relation between the potato and the enrichment, presenting the highest value for 1.85 ± 0.7 cladocerans per volume per day, in the concentrations of 30 ppm and 15 ppm respectively. The daily media density does not seem influenced by yeast addition.

The mortality rate presented significant differences in relation with yeast and enrichment nutrients. The mayor mortality was shown in the treatment with yeast 30 ppm and enrichment of 15 ppm with 0.31 ± 0.12 cladocerans per day. Also it was a highly significant difference ($p<0.01$) with 0.37 ± 0.16 using potato and enrichment nutrients in the same concentration of 15 ppm. It seems that enrichment concentrations have an impact in the mortality of adult populations of cladocerans.

About the relation of nutrients found in the growth rate, duplication time and performance, two of the three nutrients presented a highly significant relation, in the adult population. The growth rate presented its highest value with 0.38 ± 0.16 per day, in concentrations of 15 ppm and 30 ppm of potato and enrichment respectively, followed by the concentrations of 30 ppm and 15 ppm of yeast and enrichment respectively, with a maximum value of 0.36 ± 0.15 per day.

In the variable duplicative time highly significant differences were observed p (<0.01) reputedly in two nutrients, as follows: In yeast and enrichment nutrients, with 30 ppm and 15 ppm concentrations respectively, the minor duplicative time was observed with 2.18±0.7 days. Additionally, with potato and enrichment nutrient, with concentrations of 30 ppm and 15 ppm respectively, a duplicative time of 2.21± 0.9 days was presented.

8. Discussion

The present study is a experimental job that specifies the effect variability of two nutrients and enrichment media (*S. cereviseae*, potato and soy oatmeal flour) in two concentration level used (15 ppm and 30 ppm) over the population dynamics of *D. magna.*

It is known that the relative fecundity on cladocerans populations is hard to determine. Even though it is known that conditions and the food amount have an influence on this variable. Martínez-Jerónimo et al. (2008), fed *D. magna* with *Ankistrodesmus falcatus and*

Scenedesmus incrassatulus, in 6, 12 and 18 mgL concentrations and 19°C of temperature, and they observed with a mayor food concentration there was less survival and the egg number per female was on a production interval of 9 and 23, the mayor values where observed in less food concentration, agreeing with mayor fecundity. In the essay, the mayor number of egg per female in adult population and the young breeders was 5.0 ± 0.0^{a} y 3.83 ± 0.82^{a} eggs per female respectively, in treatment T6, that correspond to high concentrations of food. Similarly, the highest maximum density was observed with 15.0 ± 9.76^{a} and 8.25 ± 1.70^{a} cladocerans, and a minor population survival, under special stress conditions and plenty of food, with a room temperature of 21-25°C, showing a similar tendency on the obtained results by the previously mentioned authors in relation with the amount of food, population reproduction and survival.

A work from Hülsmann (2001), with *Daphnia galatea* showed an obvious dependency between fertility and food concentration. He found as well, a relation between the offspring maximum and the size of the body (Hülsmann, 2001). Other authors have demonstrated a clear relation between the food concentration and the number of eggs per female (Müller-Navarra &Lampert, 1996).

Data about productivity index in Daphnia magna cultures only is reported by the authors Jana & Pal (1983), whom used farmyard leftovers, caw manure and Mahua (*Madhuca indica*) substratum. Their values agree with the values originated in this research. It is important to indicate that the study of Jana & Pal (1983) was completed under normal conditions.

Sevrin-Reyssac (1993) found a production interval of *D. magna* between 200-400 g/m^3/week, feeding with micro-algae in a media of pork manure, in 2 m^3 cells during summer time (18-25 °C). During winter time they only reached 30 g/m^3/week, even though they were fed with high micro-algae concentration. In contrast the minimum value found during this research was 4.816 g/m^3/week, in a population of adult *D. magna*, under room stress conditions, feeding with *S, cereviseae,* potato and enrichment media of soy oatmeal flour with 21-25°C room temperature.

In cladocerans, food activity depends on the temperature of the concentration of the food; in concentrations of food over the threshold concentration of incorporation, the nutrition rate increases, and at the end there is a high quantity of energy available for growth and reproduction, according to Heugens (2006). Therefore a better performance of the reproductive and productive variables is expected from the populations where there are nutritional conditions of abundance and appropriate temperatures (close to 20°C) (Heugens et al; 2006).

The previous information was confirmed in this present work, where an excellent performance was presented on the growth rate, number of neonates per female, number of eggs per female, final weight, net reproduction rates obtained on T6, T7 and T8 Treatments. These treatments presented the highest concentrations of food, improving this way the averages of these variables not only for adult population but also for the young breeder population.

These facts are suggesting that the use of probiotics such as *Saccharomyces cereviceae,* soy oatmeal flour and potato for concentrations of 15 ppm and 30 ppm, as food for *Daphnia*

magna, possibly improve the biochemical composition quality as life food, since the present work reflected a better performance of this productive and reproductive variables in both populations, although they were under stress conditions. Additionally, the nutritional value of Daphnia it is not the optimal for fish post-larvae, because Daphnia does not fulfill the nutritional requirements of all the fish poslarvae (Watanabe et al., 1983). Because of this situation in the nutritional fish larvae studies made previously, is important to determine the biochemical composition of the organisms used as life food.

The duplicative time of the culture, allow us to predict the abundance of cladocerans that are able in any moment of the growth curve and it is an intrinsic characteristic of each culture under those growth conditions (Cerna et al; 2009). This concept is similar to the generational time and can be estimated using the life tables as generational time or to start from the exponential equation of growth as the duplicative time. These estimations are different, and this is because the life tables were the generation times are estimated, they do not consider the alive and death percentage on its construction and the estimation that is made for the duplicative time on the exponential equation (Werdin y Ferrero; 2008; Cerna et al; 2009).

In this essay experimental design interactions were found, with a significant relation p (<0.05) in two of the three nutrients used in the adult population; this mean, the enrichment nutrient was present on the interactions that influenced on the productive variables (daily media density, growth rate, duplicative time and performance).With the nutrients potato and yeast did not happen the same, their reflected interaction with the enrichment media, separately. This allows as confirming that one of the two nutrients yeast or potato was added unnecessarily. Excepting the variables maximum density and daily media density; where both nutrients influence notoriously. Particularly on the variables of maximum density and final weight, and which got influenced by the yeast significantly. It is possible to confirm that the use of probiotics as *S. cereviseae* improves the productivity of *Daphnia magna*.

9. Conclusions

From the diet provided to the young breeder population and the adult population, the productive variables that showed better performance were T2 and T6.

Both populations presented a poor performance on the diets with T1, were the diet supply had the minor nutrient concentration (15 ppm).

In reference to the abundance of cladocerans, Treatments T6, T7 and T8 presented a notorious performance; these treatments had the biggest nutrient concentration (30 ppm).

In the young breeder population, a better homogeneity in data from all the treatments was observed. Including the data front e treatment T6 from both populations. This suggests that to evaluate nutrients, use a neonate population with less than 24 hours.

The present work permitted compile the existing information about maintenance of zooplankton cultures, common calculations used to study the secondary production of zooplankton and the assessment of the productive and reproductive parameters on the

dynamic population of *Daphnia magna* together with the reproduction net rate and generation time, proper parameters of biological harvests with continue growth like Daphnia magna.

Although the collection of common productive variables cited in books was made, it is sufficient to evaluate some of them, for example: maximum density, growth rate and duplication time. Regarding the reproductive variables, the number of eggs per female and the age on the first offspring, reflected this performance. Also the productivity index P/B is important to calculate, because this index reflects the turnover rate of the harvest, and allow it to predict the productivity during a period of time.

The maintenance of each individual isolated avoid intra-specific and inter-specific competency for food, guaranteeing the wellness of the organisms, the best use of metabolic energy and the inadequate concentration of metabolic waste.

From the previous work we can say that yeast is a key nutrient in 30 ppm concentrations over the maximum density and the final weight, not considering the addition of enriched media, this last one did not present a significant relation ($p<0.05$). Additionally, the parameters growth rate, duplication time and performance, are highly affected by the enrichment nutrient. This let us make sure that one of this ingredients yeast or potato was added unnecessary.

It is important to design a diet with the enrichment media used on this research (soy oatmeal), keep in mind not selecting nutrients such as potato and yeast at the same time, because they present the same effect in the valuation on the adult population.

Finally *Daphnia magna* can be considered a specie with reproductive potential, because of it easy management and resistant to manipulation, even under stress conditions, this can be important in life food production in small infrastructures.

A study of pathogenicity should be made on species that uses probiotics, as well as life food.

10. Acknowledgment

The authors express their gratitude to the professor Martha Olivera for her constant support on the consecution of the equipment needed to develop this project, as well as to the zootechnician Luis Fernando Zuluaga that assisted with the translation of the document.

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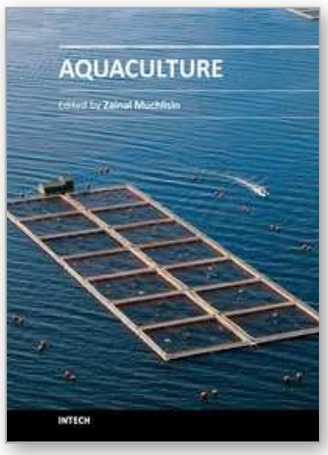

Aquaculture

Edited by Dr. Zainal Muchlisin

ISBN 978-953-307-974-5
Hard cover, 390 pages
Publisher InTech
Published online 27, January, 2012
Published in print edition January, 2012

This book provides an understanding on a large variety of aquaculture related topics. The book is organized in four sections. The first section discusses fish nutrition second section is considers the application of genetic in aquaculture; section three takes a look at current techniques for controlling lipid oxidation and melanosis in Aquaculture products. The last section is focused on culture techniques and management, ,which is the larger part of the book. The book chapters are written by leading experts in their respective areas. Therefore, I am quite confident that this book will be equally useful for students and professionals in aquaculture and biotechnology.

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Lucía E. Ocampo Q., Mónica Botero A. and Luis Fernando Restrepo (2012). Measurements Population Growth and Fecundity of Daphnia Magna to Different Levels of Nutrients Under Stress Conditions, Aquaculture, Dr. Zainal Muchlisin (Ed.), ISBN: 978-953-307-974-5, InTech, Available from: http://www.intechopen.com/books/aquaculture/measurements-population-growth-and-fecundity-a-culture-of-daphnia-magna-to-different-level-of-nutrie

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