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Research Paper

Effect of different feeding regimes on the performance of *Daphnia longispina*.

[*1]**Mona** MH, [1]**El-Gamal** MM, [2]**Abdel** Razek FA, [1]**Elgiar** EA, **Nour** Eldeen MF.

[1]Zoology Department, Faculty of Science, Tanta University, Egypt.
[2]National Institute of Fisheries Oceanography, Alexria, Egypt.

* Corresponding Author E-mail: Drmona2005@hotmail.com

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ABSTRACT

The study was conducted to evaluate the best feeding regime that will be helpful in reducing the cost of production and increasing the profit margin of *Daphnia***.** *Daphnia longispina* was collected from Kafr El-Sheikh (Egypt). Four food types were tested (*Scendesmus quadricola; Osillatoria ocutasama*, horse manure and yeast). Six criteria were investigated (Length and width; fecundity; number of offspring/brood; number of broods/female; number of offspring/female and Life span) to meet the food quality. The tested foods didn't affect the survival, swimming activity or life span of Daphnia. Daphnia fed with *Scendesmus quadricola* scored significant length, width, number of offspring/pouch, total offspring/female followed by yeast. While those fed with the other algae (*Osillatoria ocutasama*) recorded the lowest outcomes followed by horse manure.

Key words: *Daphnia*, food, quality, quantity, algae, yeast, manure, reproduction.

INTRODUCTION

The cultivation of fish and shellfish larvae under controlled hatchery conditions requires not only the development of specific culture techniques, but in most cases also the production and use of live food organisms as feed for the developing larvae. Live food organisms contain all the nutrients such as essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids and fatty acids (New, 1998) hence they are commonly known as “living capsules of nutrition”. Providing appropriate live food at proper time play a major role in achieving maximum growth survival of the young ones of finfish and shellfish. It is obviously agreed that the production of live food organisms continues to be a very important first step in intensification of aquaculture because supplemented artificial feed cannot meet all the elements required for the growth of fish (Das, 2012). The water fleas *Daphnia* (Crustacea, Cladocera) often dominate zooplankton biomass in freshwaters all over the world (Lampert, 1987). They are very efficient grazers on phytoplankton and bacteria protozoa. It is a major food source for many planktivorous fish species (Buktenica et al., 2007) therefore represents a key species in the aquatic food web. Due to their extraordinary reproductive ability, *Daphnia* populations can quickly react to changing food conditions (Müller et al., 2007). As discussed by Sterner Elser (2002), the most prominent factor that leads to a divergent response of species is the available food which can vary both in quantity and quality, influencing species metabolism directly. Many investigators have studied how food availability affects growth reproduction in the more common *Daphnia* species. Key literature prior to 1989 was reviewed by McCauley et al. (1990*a*); later publications with new data include McCauley et al. (1990*b*) Nisbet et al. (2004) added helpful information. Many literatures have focused on food quality, characterized by the stoichiometry of Key elements demonstrating the importance of Carbon: Nitrogen, Carbon: Phosphorus ratios in the algal food for *Daphnia* growth reproduction (Urabe Sterner, 2001 Sterner Elser, 2002). Others have developed dynamic models describing this phenomenon (Kooijman 2000; Nisbet et al., 2004). The models differ in their assumptions regarding priority for energy allocation; however, all models recognized in some manner increased ‘priority’ for reproduction versus growth as an animal ages. Generally low food quality is known to affect fitness in *Daphnia* adversely. (Boersma, 2000) plays an important role in explaining the community structure population dynamics (Sterner Elser 2002). *Daphnia* species are non-selective filter feeders that are not able to discriminate between different food particles (DeMott 1986), they are highly susceptible to changes in species composition. As for all consumers, also for the filter-feeder *Daphnia* the ideal food source would be easily ingestible; the digestible contains all essential compounds matching the nutritional needs of the organism at all developmental stages. Under natural conditions, these requirements are rarely met.

Daphnia often encounters food sources of sub-optimal quality causing limited growth (Nizan et al., 1986, Reinikainen et al., 1994). Several factors that determine food qualities in algae were identified. They can be classified into, size morphology (DeMott 1995), biochemical content (Müller-Navarra 1995, Sundbom Vrede 1997), nutrient content (Sterner Elser 2002) and toxicity (Jang et al., 2003).

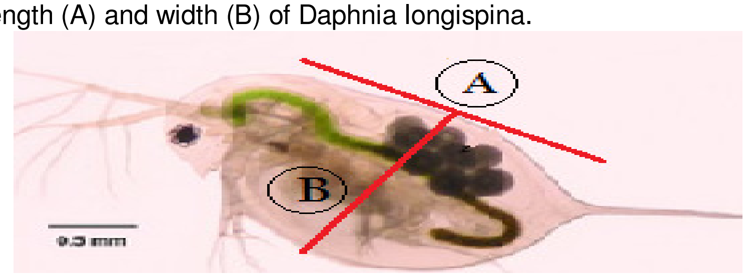
The present work was designed to increase reproductive capacity and growth of *Daphnia longispina* inhabiting Egyptian water by using different food sources (yeast, micro algae, manure and artificial diets) to reduce the cost of production thereby increasing the profit margin of *Daphnia*.

MATERIALS AND METHODS

Daphnia longispina was collected from Kafr El-Sheikh (Egypt) maintained at 24±1ºC at a controlled photoperiod of 16 hrs light and 8 hrs darkness. When starting to culture, D. *longispina*, the first cultures were initiated with 15 neonates per vessel/L freshwater. These initial neonates originate from a single female in order to minimize clonal variations such as age at maturity brood size. Future cultures were initiated with 10 neonates (<24 h old) /vessel/250 ml originating from a pool of offspring collectively produced by the parental cultures. Vessels are covered with cling-film to minimize evaporation to reduce contamination. Four food types were used (*Scendesmus quadricola, Osillatoria ocutasama*, horse manure and yeast). Semi-continuous cultures of *Scendesmus quadricola,* Osillatoria *ocutasama* algae were used according to Zehnder Gorham (1960) with sufficient phosphorus content. These cultures resulted in algal cells with a molar carbon to phosphorus (C: P) ratio of 70 - 80 for P-rich cells (P+). The concentration of used algae was 4.5 x 10^{-3} cells / ml. Horse-manure soil medium of Banta (1921) with a modification (air dried horse-manure was used instead of fresh as stipulated by Banta). This together with soil was allowed to stand in tap water for two days, and the mixture was filtered through fine silk bolting cloth. The filtrate was permitted to stand until the turbidity disappeared, which usually occurred within two days. Medium over five days old from the time of filtering was never used. Every day, the culture medium was replaced with fresh medium with a selected type amount of food for 21 days. Seven replicates for each food regime were established. Fecundity, number of offspring/female, number of offspring/brood, and number of broods/female were counted every day. Life span due to each food regime was recorded.

The activity of the tested specimens was observed and scored as the movement rate in the culture media and the quantity of food inside the alimentary tract, because ingestion rate was difficult to be measured. Core lengths (A) width were measured on *Daphnia* (Figure 1) while keeping them alive for subsequent growth production. Thus, growth rates could be assessed for the same organism. The measurements were taken under a microscope to an accuracy of 0.025mm.

Figure 1: The core body length (A) and width (B) of Daphnia longispina.



Statistical analysis

The results are presented as mean ± SD values. One-way analysis of variance (ANOVA) was used to test the significance of each food type, two ways ANOVA Tukey test to check the significant of food types with time. All statistical analysis was performed using the SPSS 15.0 software (SPSS 2006).

RESULTS

Effect of food types on the growth of *Daphnia longispina*

In statistical terms, a linear regression model yielded the best fit between age and body lengths (mm) with the four types of food as presented in figure 2. When slopes were compared, a clear pattern emerged. The daily growth rate was lower in the cultures fed with horse manure (y= 0.0903x+1.555, R^2= 0.74) followed by *Osillatoria* (Y=0.0741x+1.3, R^2=0.896), while it was faster in the case of S*cendesmus quadricola* and yeast (R^2=0.93 and 0.943) respectively. The test of Tukey showed significant differences for the length at the age of 21 days between groups fed with *S. quadricola* ($p < 0.05$) and the rest groups fed with the other food types. Considering the mean values as illustrated in figure 3, it can be observed that *Daphnia* with *S. quadricola* attained 4.03mm length 1.55 mm width at the end of the experiment (21 days) followed by horse manure (3.0 mm length and 1.23mm width) in spite of this, growth line wasn't clear as in the case of yeast or *Oscillatory ocutasama*. When *Daphnia* reached maturity at the first week the growth line became slower.

Figure 2: Growth of *Daphnia longispina* reared under different food regimes (A) *Oscillatory ocutasama* (B) *Scendesmus quadricola* (C) yeast (D) horse-manure.

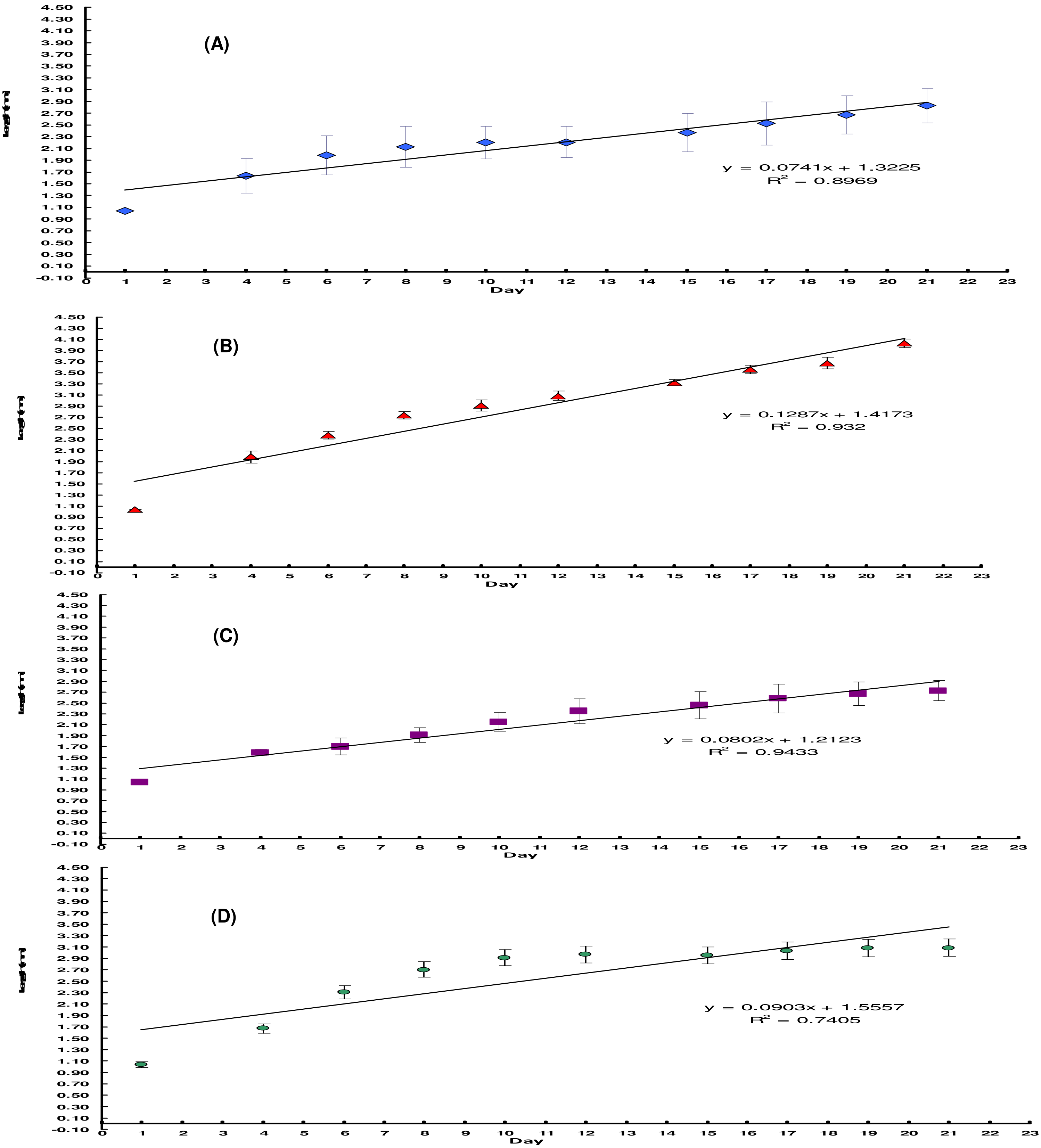
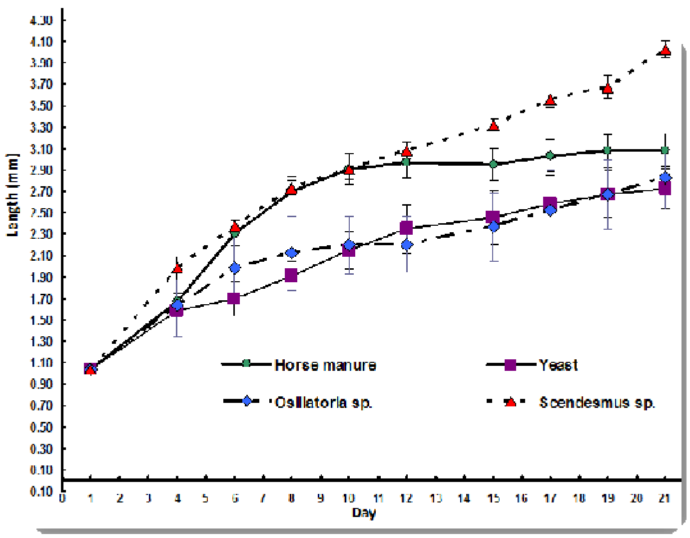
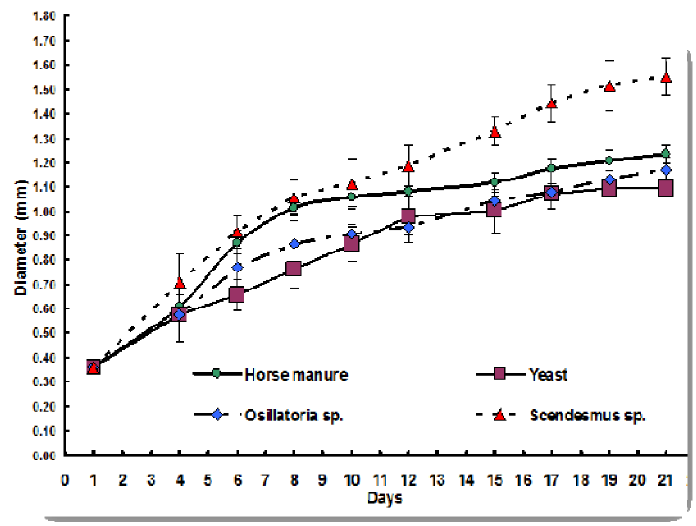


Figure (3): The effect of different food types on (A) length (B) Width of *D. longispina*.

A



B



Maturation and reproductive outputs

D. longispina reached maturity within six to seven days at 24ºC and released their eggs into a brood chamber. The embryos completed their development inside the brood chamber and hatched as free-swimming neonates at day 6-7 (table 1). In the following 2 days the mature females released a 2nd brood of neonates; the 3rd brood gave reproduction peaking around day 11-12, while the 4th brood was at day 13-14.

Table 1: Number of brood pouches offspring / female of D. longispina cultured at different food regime. Each value =Mean ± SD for 7 determinations.

Types / Day	Yeast		*Scendesmus quadricola*		*Osilatoria acutisma*		Horse manure	
	Brood pouch	Offspring No.	Brood pouch	Offspring No.	Brood pouch	Offspring No.	Brood pouch	Offspring No.
1	empty	-	empty	-	empty	-	empty	-
2	empty	-	empty	-	empty	-	empty	-
3	empty	-	empty	-	empty	-	empty	-
4	empty	-	empty	-	empty	-	empty	-
5	empty	-	empty	-	empty	-	empty	-
6	filled	-	filled	-	empty	-	empty	-
7	empty	10±0.7	empty	15±0	filled	-	filled	-
8	filled	-	filled	-	empty	10±0.4	empty	10±0.5
9	empty	10±0.5	empty	15±0.3	empty	-	filled	-
10	filled	-	filled	-	filled	-	empty	9±0.8
11	empty	9±0.5	empty	13±1.4	empty	10±0	filled	-
12	filled	-	filled	-	empty	-	empty	9±0.8
13	empty	9±0.7	empty	9±0.5	filled	-	filled	-
14	filled	-	filled	-	empty		empty	10±0.9
15	empty	10±0.3	empty	14±1.5	filled	10±3.7	filled	-
16	filled	-	filled	-	empty	-	empty	7±1.6
17	empty	10±0.7	empty	12±1.6	filled	-	filled	-
18	filled	-	filled	-	empty	9±1.0	empty	7±4.5
19	empty	10±0.7	empty	14±1.5	empty	-	empty	7±3.4
20	filled	-	filled	-	filled	-	filled	-
21	empty	10±0.3	empty	9±0.5	empty	8±1.7	empty	10±3.6
Total	8	78	8	91	6	47	7	69

The reproductive processes of females were continued till the end. As the adult *Daphniids* became older the time between broods didn't change and the size of the brood also didn't change. Four distinct periods were recognized in the life history of *Daphnia longispina*: (1) egg, (2) juvenile, (3) adolescent (4) adult. Typically, a clutch of 7 to 15 eggs were released into the brood chamber depending on the type of food. Offsprings reached their highest number (15 offspring / pouch) in the case of *Scendesmus quadricola* feeding and released ≈7 offspring/pouch in the case of horse manure feeding. On the other side, there were no significant differences (P> 0.001) between offspring numbers of *Daphnia* fed with yeast or *Osilatoria acutisma* algae both about 10±0.5 neonates. Two ways ANOVA showing non significant differences between groups of *Daphnia* fed with *Scendesmus quadricola, Osilatoria acutisma* and yeast due to the duration of the experiment (21 days) (P>0.01). While in the case of horse manure feeding, it was significanty decreased with time. The total offspring/female/21 days are shown in figure 4, it was significantly increased in groups fed with *Scendesmus quadricola* (P<0.1) as analysed by one way ANOVA compared to the other groups, it recorded ≈95 offspring. Yeast as a food stimulated *Daphnia* to release ≈78 offsprings/21 days, followed by horse manure (69 offspring) finally *Osilatoria acutisma* recorded the lowest outcomes of the offspring (47 offspring).
The number of brood pouches/female/21 days ranged from 6-8 broods. No food type altered the mode of reproduction, and no ephippiia were observed during the experimental time (21 days) till the life span (45-47 days) as mentioned in figure 5.

Figure 4: Total offspring/female/21 days of *D. longispina* under different food regimes.

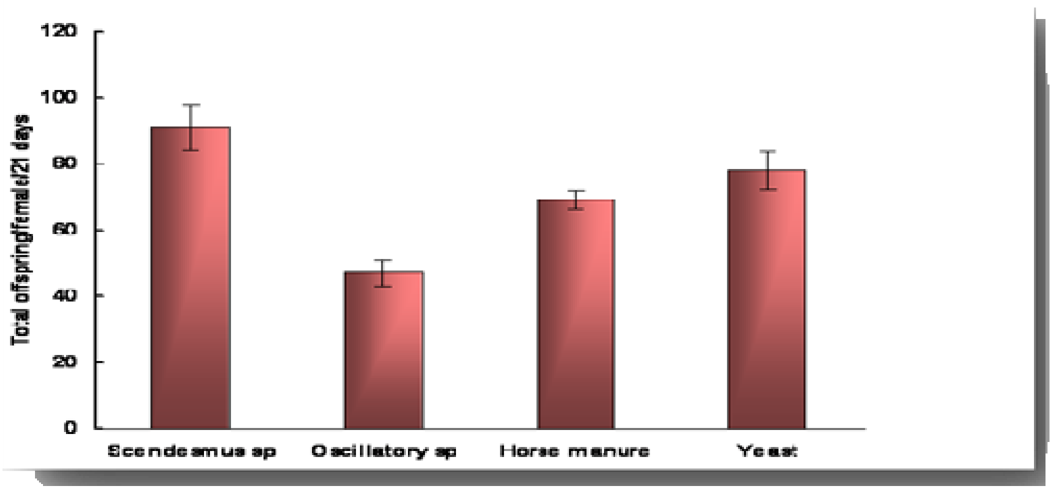
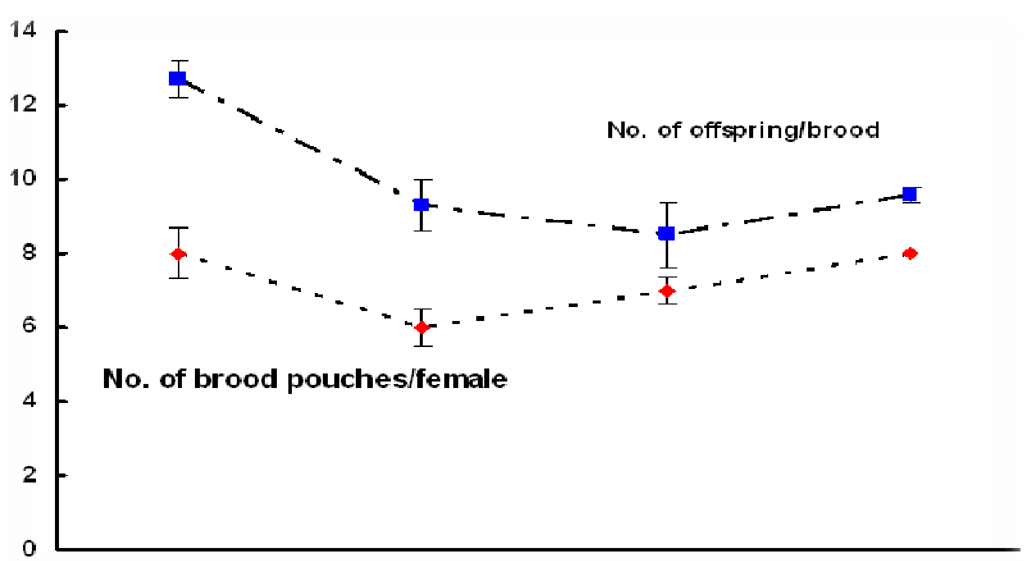


Figure 5: The number of offspring/brood number of brood pouches/female of *D. longispina* feeding different food types.



Life span

The life span of *D. longispina*, from the release of the egg into the brood chamber till the death of the adult was not depending on the type of food. One way ANOVA test illustrates none significantly differences (P<0001) between the life span and the investigated foods; it was 45± 2 days at 24°C.

Survival

There was record of death (100% survival) with all groups of *Daphnia* cultured under the four food regimes till the end of experiment (21 days).

Activity

No effect of type of food on the swimming activity of *Daphnia*, all cultures loaded with active grazing females.

DISCUSSION

As for all consumers, also for the filter-feeder *Daphnia* the ideal food source would be easily ingestible; digestible contains all essential compounds matching the nutritional dems of the organism at all developmental stages. Under natural conditions, these requirements are rarely met; *Daphnia* often encounters food sources of sub-optimal quality causing limited growth as discussed by Mitchell Lampert (2000). In the herein experiment, there were no large variations in the shape of *Daphnia* cultured at different feeding regime except differences in the size of *Daphnia* fed with *Scenedesmus*. In nature, variation in size shape within *Daphnia* species has generally been considered to be adaptive (Jacobs 1987; Dodson, 1989). As individuals grow and mature, they may experience different selective pressure and adjust their morphology accordingly. For example, it has been argued that predators selecting *Daphnia* of different sizes may induce various morphological defences such as spines, helmets neck teeth (Walls and Ketola, 1989). As such defences may entail energetic costs expressed in *Daphnia* of different sizes. The growth rate of the investigated species under any food regimes was linear over the time, the same is observed in many species of cladocerans (Taylor, 1985; Perrin, 1989). Also, when individuals reached maturity, growth was unhurried. The previous finding compromising with Frey Hann (1985), they set up that as animals become older, growth rates usually decline with size, they may eventually reach a stable size. It typically invest most of their energy in reproduction (*D. magna* 69%, *D. pulex* 67%), while they invest comparatively little on growth (23%). *Daphnia* reared with *Scendesmus quadricola* attained larger size (length and width) than the other types of food in all experiments followed by Yeast. The same is concord with the results of Hall (1964) Vijverberg (1989) who suggest that there is a strong relationship between body length and the food type. The advances on the growth were paralleled with the highest number of offspring. Rocha (1983) found a strong correlation between the body size of the mother and its offspring. Rocha emphasized that the size of the body can be a limiting factor to the number of eggs and embryos carried by the mother when food is abundant. This relationship is also observed in *D. similis* (Pedrozo Bohrer, 2003), the larger the females, the higher the production of neonates. When females of the herein *Daphnia* reached the largest adult size (3.5 mm), it also presented the highest number of neonates. The data of Heugens, et al. (2005) revealed that food is the important factor that influences life-history parameters of *Daphnia.* The last assumption wasn't clearly noticeable with the present investigation, because the life span of *Daphnia longispina*, under the tested foods was not significantly different and it recorded ≈45 days at 24^{o}C. Pennak (1978) found that life span of *Daphnia* is highly variable depending on the species environmental conditions. He recorded average life span of *D. magna* as about 40 days at 25^{o}C for *D. pulex* about 50 days at 20^{o}C. In the attendance species (*D. longispina*) four distinct periods were recognized in the life history of *Daphnia*, the same is postulated by Pennak (1978) as (1) egg, (2) juvenile, (3) adolescent and (4) adult. He found that the time required to reach maturity varies from 6 to 10 days. The previous finding was broad because maturation time in tested species under any food types didn't exceed more than 6 days.

Several factors that determine food quality in algae are identified and can be classified in size morphology (De Mott 1995), biochemical content (Sundbom Vrede 1997), nutrient content (e.g. Carbon: Phosphorus or: Nitrogen) (Sterner Elser 2002) and toxicity (Jang et al. 2003). For all variables that determine the quality of a food resource, phosphorus has been suggested to be the main limiting nutrient (Schindler 1978). In *Daphnia* there was a significant impact of food nutrient content even at low food levels (Sterner Robinson, 1994), attention has also been turned to potential interactions between food quality and food quantity as the potential role of nutrient limitation (Sterner Elser, 2002). In the present study concerning food quality, *D. longispina* performed best on a diet of *Scendesmus quadricola* while the second type of algae (*Osilatoria acutism*a) didn't record the same outcomes. Taub and Dollar (1964) concluded that *D. pulex* did not reproduce normally when fed with *Chlorella pirenoidosa* and *Chlamydomonas reinhardii*. It showed shorter longevity, ovulation and the embryos did not complete their development. In other study on the food quality of freshwater phytoplankton for the production of cladocerans, it was found that from the spectrum blue-greens, flagellates green algae, *Daphnia* performed best on a diet of *Rhodomonasminuta Cryptomonas* sp., they correlate this upshot to the high levels of HUFA of these algae (Reinikainen et al., 1994).

Effects of the green alga *Scenedesmus quadricola* and the filamentous cyanobacterium *Oscillatoria limnetica* as food sources on the growth reproduction of nine *Daphnia galeata* clones were studied by Repka (1997). Although *Oscillatoria* is regarded as a low quality food for *Daphnia* because of its filamentous morphology suspected nutritional inadequacy, the cyanobacterium sustained both body growth and reproduction in *D. galeata.* The horse manure food didn't offer good result with the present *D. longispina* especially in reproductive outputs. This result is contradictory with the finding of Jana and Pal (1985), they compared six different culture media for *D. carinata*, and they found that in medium enriched with cattle manure the animals presented highly increased abundance production. Other researches confirmed the present finding in the role of algae as a luxury food for *Daphnia*. Cowgill et al. (1985) observed that the animals that fed with algae are more longevous than animals fed with other diet including artificial ones. Schwartz Ballinger (1980) was realistic that the algal species used as food for *D. pulex* influenced its survival, age to maturation and number of their clutches.

CONCLUSION

The green alga *Scendesmus Quadricola* is the best food for *D. longispina* to attain high productivity.

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