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Salinity effects on survival and life history of *Daphnia magna*

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Abstract: Salinity is one of the most significant problems that the environment of Iraqi freshwater faces where the suffering of the Tigris, Euphrates and the Shatt al-Arab Rivers as well as land in the past with only the finest of the clear increase in the quantity of salts. There is relatively a little information available on the responses and adaptations of freshwater organisms penetrating into brackish water. Bearing this in mind, we studied the acute and chronic effects of different salinity values in: *Daphnia magna* Straus, Salinity experiments were based on successive dilutions of a stock solution of coarse salt was collected from the land of Wasit Governorate-Iraq, The mortality was significantly affected by the interactive effect of suspended bentonite clay Salinity significantly affected all physiological variables on both experimental occasions. Total protein slightly increased for *Daphnia magna* reared in salinity 0.5‰, 1‰, 1.5‰, than those reared in control group.

Keywords: Toxicity tests, Coarse salt *Daphnia magna*, life history

Introduction

Salinity intrusion in freshwater ecosystems is a problem that has become increasingly serious due to environmental change. These systems suffer from important alterations with the increase of salinity due to global climate change, such as the action of rainwater, wind, evaporation and sea level rise (Schallenberg *et al*., 2003). Salinity is one of the most significant problems that the environment of Iraqi fresh water faces where the suffering of the Tigris, Euphrates and the Shatt al-Arab as well as land in the past with only the finest of the clear increase in the quantity of salts. Several authors (Hall and Burns, 2003, Schallenberg *et al*., 2003,) have reported there is a reduction in the diversity and abundance of species communities exposed to salinity increases. Freshwater species require completely different adaptations to maintain suitable osmotic pressure and cell homeostasis, most aquatic organisms are unambiguously characteristic of either one habitat or another (Arner and Koivisto, 1993).

Studies have been carried out in freshwater ecosystems threatened by salinity in order to evaluate how species, altering the ecosystem structure, and function are affected by the rise in salinity affects (Hall and Burns, 2003, Nielsen *et al*., 2003, Schallenberg *et al*., 2003, Rokneddine and Chentoufi, 2004). Still, there is a little information on how the increase of salinity affects ecosystem integrity so as to predict the impact and rehabilitate aquatic systems (Nielsen *et al*., 2003). Therefore, it is important to study the effect of salinity stress in freshwater organisms. Among these, zooplankton has long been used as a suitable group to assess the impact of environmental change, in part due to its key intermediate position in the trophic food web. Zooplanktonic organisms are frequently used as indicators of water quality due to the close relationship between environmental factors and species composition (Barros *et al*., 2007), which are mainly affected by mineralization gradients (Sanchez-Colomer, 1996 , Boronat *et al*., 2001, Amsinck *et al*., 2003). Salinity changes in freshwater systems may greatly affect the survival and life history (growth and reproduction) of zooplankters and, consequently, have an impact on higher trophic levels. Cladocerans, one of the most abundant primary consumer groups in lentic and lotic ecosystems, show different physiological tolerances to several ionic components (Bailey *et al*., 2004). The genus *Daphnia* is freshwater in its origin and distribution (Teschner, 1995) for North America, there are 34 species in freshwater environments and only one for saline lakes, *Daphnia salina* (Hebert *et al*., 2002). In previous studies, also demonstrated that *Daphnia magna* is essentially recognized as a freshwater cladoceran, but there are some strains that grow in brackish waters. In Iraq many cladoceran species were listed from several parts, (Ajeel *et al*., 1997, Ajeel and Abbas, 2012, Al-Seria and Jaweir,

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2015).There is relatively little information available on the responses and adaptations of freshwater organisms penetrating into brackish water.

The aim of this study is to investigate the effect of salinity of each of rock saline as a model compound, on survival, growth and reproduction of *D. magna* and to establish the maximum salinity level in which *Daphnia* can survive and reproduce to be used as a natural food in aquaculture where *D. magna* is of high nutritive value for aquatic animals.

Materials and Methods

Experimental Animals and Food

A freshwater *D. magna* strain that has been successfully grown in the laboratory of Biology Department, College of Science for Women, University of Baghdad for more than 5 years in synthetic freshwater media that was used as the test organism for this study. Gravid females were moved at regular intervals to 1 liter glass beakers, in which the culture medium, synthetic freshwater medium (pH, 7.2). In these beakers, the animals were fed 3 times a week with $14x10^5$ individual/ml of *Scenedesmus obliquus*. It was previously determined that this cell concentration is an optimal food dosage for this strain. The algal culture was renewed once a week to maintain the algae solution in good condition. The algae and the daphnids were kept at a temperature 22 ± 2°C with a light period of 16 L: 8 D both during culturing and experimental periods. Salt was collected from Sa'id ibn Jubayr district in the district and Badra in the Wasit Governorate (Fig. 1) and analyzed the salt by x-Ray that have been briefly described in Table 1.

Fig. 1: Sampling area (Wasit Governorate-Iraq).

Tab. 1: Analysis of rock salt.

Salt	Percentage
NaCL	88
Al_2O_3	5
Na_2CO_3	3
MgO	2
K_2O	1
Others	0.91

Acute tests

Acute toxicity testings were in triplicates where groups of 10 < 24 h-old daphnids are placed in 250- ml beakers, each containing 100 ml medium and subjected to test conditions for 48 h. Tests were run without food addition. The number of live organisms after the elapse of 48h is recorded. Control test is run in runs. Salinities series 0.75, 1, 1.5, 1.75, 2, 3, 4, 5, 6, 7, 8 and 9‰ (parts per thousand, ppt). 50 mg of suspended bentonite clay was used per liter of water and this water was used in experiments. All practical experiments were based on the endpoint where the immobilizing animals at the bottom of the baker were adopted.

Chronic tests

Ten neonates (<24h-old, standard length of 1.60 to 2.00 mm) were placed in each 250 ml-glass beakers containing 100 ml of synthetic freshwater for control or saline water for each treatment which were renewed with the addition of fresh food three times a week. These experiments lasted for daphani death. Tests were run with food addition, three times a week during changing test water. Salinities effects on daphnids was investigated at series: 0.5, 1, 1.5, 2 and 3‰ for rock salt. The second experiment used bentonite (clay) and the same concentrations for rock salt. For chronic tests, three times a week, *Daphnia* were removed from their container and placed immediately into a new prepared synthetic freshwater media, as control and different salinity-adjusted treatments containing algal food, *Scenedesmus obliquus* at $14x10^5$ individual/ml. Survival, growth and reproducetion rates of daphnids were recorded three times a week. The survival rate was calculated by dividing the numbers counted every time by the number of neonates at the beginning of the experiment. Growth was determined from the body lengths which were measured under the microscope with an ocular micrometer (160 × magnification) from base of the caudal spine to the anterior edge of the head. They were measured under the microscope with an ocular micrometer (160 × magnification) from base of the caudal spine to the anterior edge of the head. Growth is described as the increase in body length over time. Growth in crustaceans is a discontinuous process, i.e. the succession of molts (exuvia, ecdyses) is separated by intermoult periods. Each time an individual moults, the old integument is shed and a rapid, extensive growth occurs during the short period before the standard length at subsequent moults was tested in function of salinity using a repeated measures analysis of variance (ANOVA). The age at release of first brood was noted. After every reproduction the offspring were counted and taken away until end of experiment to calculate the number of progeny per *D. magna* female.

Biochemical analysis of *D. magna*

Protein content of daphnids was determined according to lowry method (lowry *et al*. 1951). Lipid content was determined according to the method of Knight *et al*. (1972).

The Statistical Analysis System- SAS (2012) program was used to effect of different factors in study parameters least significant difference –LSD test and Duncan multiple range (ANOVA) was used to signify-cant compare between means in this study.

Results and Discussion

The mortality was significantly affected by the interactive effect of suspended bentonite and salinity (Tab. 2). The results of the present study show that the presence of suspended bentonite reduces the effect of salinity on *D. magna*. In general, the probit estimated the percentage of mortality that was 100% at 9‰ after 48 hours of exposure time, while mortality was 100% at 6 after 24 hours of exposure time in absence suspended bentonite and did not get mortality at low salinity (0.75, 1 and 1.5‰, with suspended bentonite).

The lethal median concentration, while the24h, 48h-LC50 for salt with suspended bentonite was 5.4 and 4.8‰ respectively, and the tested concentrations ranged from 2.3 and 1.8‰ during 24h, 48h respectively for salinity without suspended bentonite (Figs 2-5).

Table 3 shows that the safe concentration was the highest when tested with suspended bentonite than without it. It was 1.3, 1.2 at 2, 3‰ respectively. The researchers noted (Hartman and Martin 1985) that suspended solid in water, especially those with a surface activity of adsorption, caused the occupancy of a certain proportion of molecules in the form of adsorbed, which reduces the value of the deadly medium concentration. The results of the Tan and Wang study (2009) indicate that the concentration of calcium ion in the animal environment negatively affects the process of excretion, thus collecting waste and the death of the exposed animal.

Tab. 2: Effect of concentration of coarse salt suspended bentonite Clay and without suspended bentonite in dead percentage.

Con. of	With sus. b.		Without sus. b.		LSD
coarse salt	24 hr.	48 hr.	24 hr.	48 hr.	value
0	0	0	0	0	0.00^{NS}
0.75	0	0	0	0	0.00^{NS}
1	0	0	0	0	0.00^{NS}
1.5	0	0	10	20	4.25*
1.75	0	20	20	40	5.72*
2	10	20	40	50	5.47*
3	10	30	60	80	7.62*
4	20	40	70	90	7.41*
5	40	40	80	90	7.08*
6	60	50	90	100	5.96*
7	60	70	100	-	7.12*
8	80	80	-	-	0.00^{NS}
9	90	100	-	-	4.33*
LSD value	8.41*	9.57*	8.41*	8.55*	-

sus. b.: suspended bentonite
Con.: Concentration
* (P<0.05), NS: Non-Significant.

Tab. 3: Safe concentration of salinity (‰) of *Daphnia magna*.

Treatment	Safe Con. at X=2	Safe Con. at X=3
With sus. b.	1.3	1.2
Without sus. b.	2	3

sus. b.: suspended bentonite
Con.: Concentration

The study of Ghazy *et al*. (2009) showed that the value of LC50 differed by source of salinity. Exposure of *D. magna* females to concentrations of table salt, sea water and natural sea water resulted in LC50 variation after 48 hours of exposure, reaching 2.99, 3.92 and 4.82%, respectively. While the results of the study of Lilley (2002) indicated that the value of LC50 goes up to 3.3 after ٤٨ hours of the exposure of *D. magna* females to different concentrations of sodium chloride salt. The result of this may lead to the occurrence of Osmosis shock due to increased salt concentration in the blood of the exposed animal (Grzesiuk and Mikulski, 2006). This corresponds to what was found by Bailey *et al*. (2004) that increasing the percentage of *Daphnia* sp. losses when transported from fresh water to brackish water.

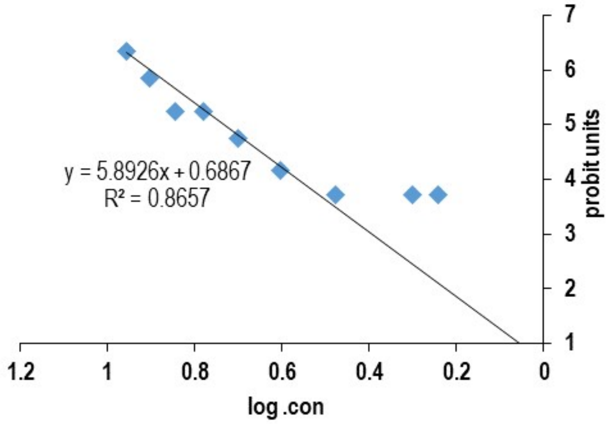

Fig. 2: Median lethal concentration (LC50) of coarse salt with suspended bentonite Clay after 24h.

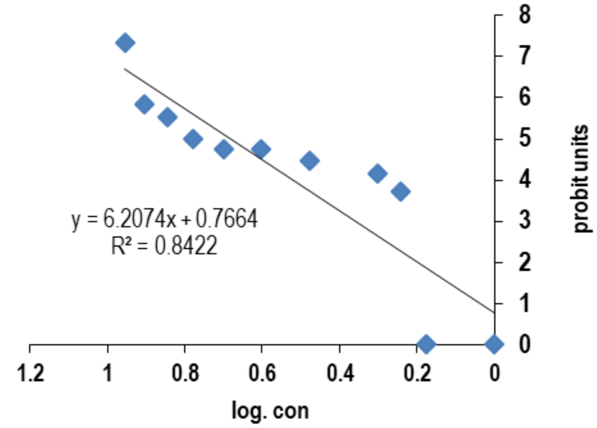

Fig. 3: Median lethal concentration (LC50) of coarse salt with suspended bentonite Clay after 48hr.

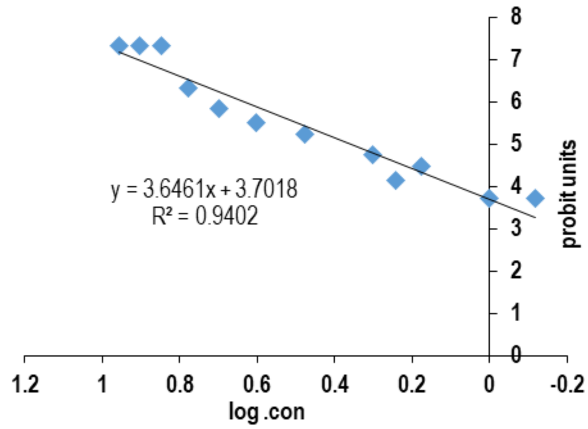

Fig. 4: Median lethal concentration (LC50) of coarse salt without suspended bentonite Clay after 24h.

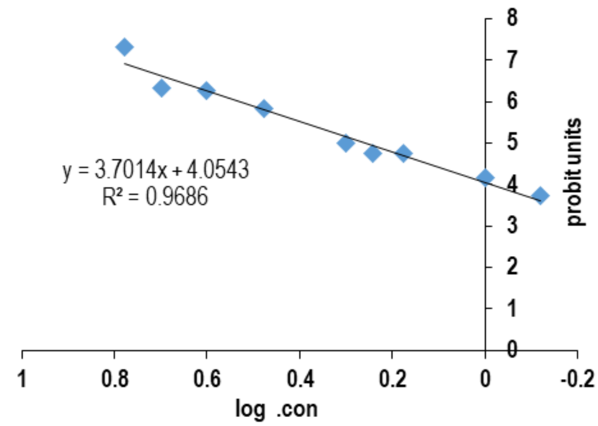

Fig. 5: Median lethal concentration (LC50) of coarse salt without suspended bentonite Clay after 48h.

The effect of coarse salt with suspended bentonite Clay on survival, growth and reproduction rate of *D. magna*. Salinity significantly affected all physiological variables on both experimental occasions (Tabs. 4 and 5). Generally all measured parameters decreased with increased concentration, the mean longevity decreased with increasing the concentrations of salinity. The highest longevity is 35 for control groups followed by those cultured in 0.5‰ without suspended bentonite.The animals were biggest in 0.5‰ (with suspended bentonite). It was found that the highest lengths and weights which represented by 5.0 mm, 9.7mg were observed for group reared in 0.5 with suspended bentonite which differ significantly than those reared in other concentrations. While the lowest length and weight were found for those reared at the highest concentration 3‰ (without suspended bentonite) represented by 2 mm and 6.2 mg respectively.

The decrease in the longevity of females under study with increasing saline concentration may be due to the high sensitivity of the *Daphnia* to salt, which has affected the composition and function of the living membranes, leading to a lack of ionic balance between and inside the body, thus affecting the weak tolerance of exposed animals due to accumulation High salinity in the blood, leading to the occurrence of poisoning (Lahdes and Karjala, 2007, Cieluch *et al*., 2004).

The results of this study are consistent with those of Shehab (1977) and Arner and Koivisto (1993). The results of their study confirmed that the growth rates of *Monia micrura* increased with an increase of saline concentrations from 0.47 to 4.13 for pond water, and from 0.363 to 5.142 for seawater. The same is true for the growth rate of type *D. magna* when salinity is increased from 4 to 8.

The results of studies of Kefford *et al*. (2007) and Ghazy *et al*. (2009) agreed upon the nature of the extreme identical relationship between the increase in

Tab. 4: The effect of coarse salt with suspended bentonite Clay on survival, growth and reproduction rate of *D. magna*.

Concentration (‰)	A	B	C	D	E	F	G
0.5	9.7a	5.0a	30a	7a	6.5b	5a	59a
1	8.5ab	4.5a	28a	5b	7b	4ab	55a
1.5	7.6b	4.2ab	27a	5b	8ab	4ab	30b
2	6.8b	3.2ab	17b	3c	9a	3bc	4c
3	6.5b	2.5b	14b	2c	10a	2c	-
LSD value	2.007*	1.71*	4.92*	1.63*	2.55*	1.82*	7.29*

A: Wet weight (mm)
B: Growth rate (mm)
C: Mean of females longevity (day)
D: Mean number of moults female
E: Age at first reproduction (days)
F: Mean number of broods female
G: Mean number of juveniles/ female
* ($P<0.05$)
Means having with the different letters in same column differed significantly.

Tab. 5: The effect of coarse salt without suspended bentonite Clay on survival, growth and reproduction rate of *D. magna*.

Concentration (‰)	A	B	C	D	E	F	G
Control	8.1^{ab}	4.3^{a}	35^{b}	5.8^{a}	7^{b}	4^{a}	49^{a}
0.5	9.1^{a}	4.5^{a}	44^{a}	6^{a}	7.5^{ab}	4^{a}	50^{a}
1	7.6^{ab}	4.2^{a}	35^{b}	4^{ab}	8^{ab}	3.5^{ab}	30.9^{b}
1.5	6.1^{b}	3.5^{ab}	22^{c}	4^{ab}	8^{ab}	4^{a}	22.2^{c}
2	6.4^{b}	2.5^{ab}	21^{c}	3^{b}	8.5^{ab}	3^{ab}	18.2^{d}
3	6.2^{b}	2^{b}	22^{c}	2^{b}	9^{a}	2^{b}	2^{e}
LSD value	2.28*	1.83*	6.71*	2.39*	1.74*	1.53*	5.87*

A: Wet weight (mm)
B: Growth rate (mm)
C: Mean of females longevity (day)
D: Mean number of moults/ female
E: Age at first reproduction (days)
F: Mean number of broods/ female
G: Mean number of juveniles/ female
*($P<0.05$)
Means having with the different letters in same column differed significantly.

salinity concentrations and the increase in exposure time on the one hand and the atrophy of growth rates on the other, where the growth rate of S. *vitulus* decreased after 16 days of sodium chloride concentration, the rate of growth of female *D. magna* was reduced to 3.38 mm in saline concentration 2.66 after 3.74 mm in the control treatment, while the study of al-Azzawi (2008) showed that the female growth rate of type *D. pulex* decreased from 2.1 mm in the control treatment to 1.70 mm in 2% concentration of sodium chloride salt.

Salinity is a determinant factor for the growth of fresh water environment. That is why growth rates for both species have decreased by increasing salinity concentration. This is confirmed by the results of Teschner (1995) Burns (2002), where the growth of the species *D. magna* and *D. carinata* slowed down when transferred from the freshwater environment to the saline water environment.

It was found that the number of moults decreased with increasing the concentration of salinity, the highest number of moults is (7 and 6) noticed for o.5‰ (without suspended bentonite, with suspended bentonite respectively, and the lowest number of moults was found for those reared at highest concentration, 3 represented by 2‰ in illustrating the effect of different concentrations of reproductive rate of *D. magna*. In this work it is shown that the concentration of 05 increased the reproductive rate of *D. magna.*

The results of the current study indicate the negative effect of salinity in reducing the rate of relapses of both types. This may be due to the effect of high concentrations of salt in inhibiting the hormones stimulating the dissociation, thereby reducing the rate of the number of offspring of females *Simocephalus vetulus* and *D. pulex* (Hirano *et al*., 2004).

Generally the age at first reproduction was 6.5 days in 0.5‰ whit suspended bentonite but was 7 days for control, while the age at first reproduction was delayed to 10 days in groups reared at 3.

The results of the present study show the gradual delay in the days required for the arrival of Age at first reproduction with the increase of saline concentrations used. This may be due to the high effect of saline concentrations in reducing the energy contained in the body through the exhaustion of most of them in the mechanism of osmotic regulation and thus neglect the reproductive aspects as proved by Hanazato (1996) and Ismail *et al*. (2010). The results of their study indicated that salinity has the obvious effect in prolonging the time required for the arrival of the organism to puberty due to decreasing metabolic rates as a reaction to external pressure which result in the decrease of oxygen and body energy and thus negatively affect puberty. Salinity affected reproducetion of females during the experiments. The number of broods/female was the highest (5) at 0.5‰ and the lowest (2) at 3‰.

Daphnia species responses appear to differ from each other. Martinesz-Jeronimo and Martinez-Jeronimo (2006) found that the number of broods/ female type of *D. magna* decreased from 16.4 broods/female in the control treatment to broods/ female at the concentration of 7‰ of salt Sodium chloride. While Arner and Koivisto (1993) explained that the reason was the apparent salinity effect in rapid breathing, offset by a reduction in the rate of ammonia elimination and thus metabolic dysfunction, negatively affecting the growth rate.

While the mean number of juveniles /female was the highest at 0.5‰ whit suspended bentonite and the lowest at 3‰. Also, the microscopic examination showed that the *D. magna* came from the dormant eggs (ephippia) when the salt concentration increased as shown in the Figure 6.

The findings of the present study are consistent with Arner and Koivisto (1993), who indicated that the number of juveniles /female *D. magna* has decreased by 18.5 g in sodium chloride salt to 12.6 g in saline concentration. The results of this study are also consistent with the results of the study of Ghazy *et al* (2009) who noted that the number of female dwarfs *D. magna* decreased from 54 juveniles /female to 32 juveniles /female when salinity was increased from 1.67 to 2.66. The decrease in the number of juveniles /female in the two types under study may be due to the salinity effect in inhibiting the ovary enzyme and thus inhibiting adolescent productivity (Gaudy *et al*., 1982). The cause of the decrease may also be due to the salinity effect of the Moulting Inhibition Hormone (MIH) inhibitor, thus inhibiting Juveniles Hormone as a result of the close relationship between the growth rate and the rate of immature females (Hirano *et al*., 2004).

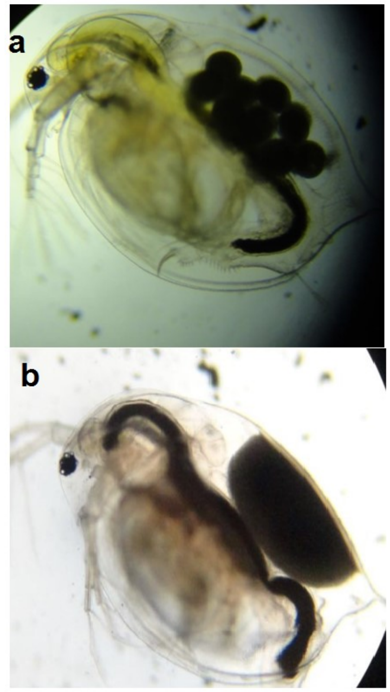

Fig. 6: *Daphnia magna*: (a) normal eggs, (b) Dormant eggs (ephippia).

Biochemical composition of *D. magna*

The protein, lipid content of *D. magna* reared in 0 and 0.5, 1, 1.5, 2‰ (Tab. 6) Total protein slightly increased for *D. magna* reared in salinity 0.5‰ , 1‰, 1.5‰ , than those reared in control group , it was 16.06 g/ 100g wet weight ,18.56 g/ 100g wet weight , and 9.9 g/ 100g wet weight restrictively, but at the 2‰ treatment the total lipid and protein were reduced ,it was 4 g/100g wet weight , 5.06 g/ 100g wet weight respectively. In general this work found that the 1‰ treatment was the highest total lipid and protein than all treatment was 30 g/ 100g wet weigh, 18.56 g/ 100g wet weight, respectively. The present investigation showed that protein and lipid content of both groups of *D. magna* which is higher than that reported earlier by (Tay *et al*., 1991, Ghazy *et al* 2007) which may be due to analytical methods used. On the other hand, the highest lipid content of 20.03% was observed for those enriched with cod liver oil emulsion and the difference between this study and our investigation may be due to the difference in the type of foods and enriched process (Watanabe *et al*., 1982, Leger *et al*., 1987). In this respect, Macedo and Pinto-Coelho

(2001) observed that the lipid level of Moina varied from 11.4% to 19.9% and this is due to feeding effect of different algal diets. In addition, Mitra *et al*. (2007) recorded lipid content in mixed zooplankton from different ponds varied from 10.79 to 14.55% DM (dry matter) and were inversely related to water temperature. Watanabe *et al*. (1983) analyzed various zooplankton, *Daphnia* containing 13% and *Moina* 12-27% lipids whereas in *D. carinata* and *Moina australiensis* it ranged from 7.29-7.73% (Kibria *et al*., 1999).

Tab. 6: Effect of Concentrations in total protein and total lipid of *Daphnia magna*

Concentrations (‰)	Total protein	Total lipid
0	9.901[cd]	9.6[e]
0.1	16.06[b]	14.6[cd]
0.25	12.72[c]	12[de]
0.5	7.281[d]	22[b]
1	18.56[a]	30[a]
LSD value	3.049*	3.827*

* (P<0.05)
Means having with the different letters in same column differed significantly.

As daphniids have a continuous need for Ca, which they acquire via active uptake, the metabolic demands of Ca acquisition may rise to levels that threaten survival when Ca availability is low. In a low Ca environment, daphniids may also be unable to build a fully functional exoskeleton, which is necessary for mobility, homeostasis and protection against predation and other. The minimum Ca concentration required for the survival of clone of *D. pulex* was between 0.1 and 0.5 mg (Rukke, 2002).

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