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Laboratory studies on developmental stages and life cycle of *Daphnia magna*

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Abstract

The freshwater cladoceran, *Daphnia magna* is of great importance being used as a key model in ecological, developmental and ecotoxicological studies. *D. magna* were collected from village ponds (Punjab), identified and then cultured under laboratory conditions. Biological characteristics like developmental period of juvenile instars or pre-adult instars, number of egg clutch/brood, body size of juveniles and adult were studied. Female *D. magna* showed the capacity to carry clutch of 5-10 eggs in her brood chamber. Adult female was found to produce clutch of eggs every 3-5 day during her life span. Before achieving adult size they undergoes 5-6 moltings i.e. gradually increases in their size. The developmental period from 1st juvenile instar till the emergence of adult varied from 6-8 days in case of females. Whereas it took 4-6 days to convert juvenile instar into adult in case of male *Daphnia*. Females attain their sexual maturity within 6-10 days. Life cycle i.e. from egg to adult was completed within 9-11 days. Overall life span of single *D. magna* was 30-45 days under ideal laboratory conditions.

Keywords: Cladoceran, *Daphnia magna*, Developmental stages, Life cycle

1. Introduction

Cladocerans are tiny aquatic crustaceans and popularly known as "water fleas." They vary in size from 0.2 to 6 mm. About 600 species of fresh water cladocerans have been reported to occur throughout the world (Korovchinsky, 2004) ^[10]. Among cladocerans, genus *Daphnia* is a keystone pelagic filter feeders found in temporary pools and large permanent lakes as reported by Cousyn *et al.*, 2001 ^[3]. *Daphnia* spp. are highly responsive against pollutants and can even react to very low concentration of contaminants, hence serve as good biological indicators of aquatic habitat and also act as biomarker for water quality assessment for fish production (Pradhan, 2008) ^[13]. During favourable conditions, *Daphnia magna* undergoes asexual mode of reproduction and producing only females in their population. A female can release a clutch of 6 to 10 eggs into her brood chamber. However under unfavourable conditions such as variation in temperature, high population density, less food availability etc. they undergo sexual mode of reproduction, resulting into production of males and production of resting eggs (Haney, 2010) ^[9]. *D. magna* act as model organism in various disciplines such as aquatic ecology and biomedical sciences due to its abundance, ease of handling, cyclic parthenogenetic life cycle and short generation time (Seda, 2007) ^[15]. In addition, it may strongly affect the mosquito population by suppressing their oviposition, increasing time to pupation and reducing total abundance of mosquito larvae via food competition (Duquesne *et al.*, 2011) ^[5]. Another interesting feature of *D. magna* is that they can easily adapt to the breeding sites of mosquitoes and shows close interaction with mosquito larval population, which makes them potential competitors against mosquitoes (Kumar and Hwang, 2006) ^[11]. Thus, the present study was aimed to study the life cycle of *D. magna* including various stages of its development.

2. Materials and methods

Water samples were collected from the selected village ponds of Ludhiana (Punjab) with the help of zooplankton net (mesh size 60 µm) by sieving 50L of water at 5 sites/village pond (approx. 10L/site). From collected water samples *D. magna* was identified on the basis of their morphological features following the standard keys given by Battish (1992) ^[11] and Haney *et al* (1987) ^[8]. Out of the collected water samples two types of females were recognised, one having parthenogenetic eggs carrying inside its brood chamber and another having resting eggs enclosed inside the ephippium (Fig. 1 a, b). Females having parthenogenetic eggs were further used for the start up culture.

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Single ovigerous female, *D. magna* was picked up and put in 250 ml of de-chlorinated water having 20 ml *Paramecium* culture along with 0.5 ml yeast stock solution. The whole setup was kept in BOD incubator set at 22 ± 1 °C temperature. For preparing *Paramecium* culture, 500 ml pond water was taken into conical flask and boiled for 10 minutes. Then 2-4 wheat grains were added to the boiled pond water and kept for 1 to 2 days. This culture media was inoculated with one or two table spoons of pond water having protozoans and kept in BOD incubator at 22 ± 1 °C temperature. Yeast stock solution was prepared using dry baker's yeast (10mg in 100ml of distilled water). The culture was checked regularly under microscope and after getting their good population the culture was shifted to large containers.

The life cycle of *D. magna* was studied by recording the development of juvenile instars/pre-adult instars till the formation of adults. Ten freshly formed females taken from pure culture were kept separately in 500ml beakers in BOD incubator at 22 ± 1 °C temperature. The size of these females i.e. length and width was measured. The formation of brood chamber of all females was observed daily and their number was recorded. After hatching of juveniles from brood chamber, the females were removed from the test culture and kept in separate containers. The newly emerged juveniles were observed daily under binocular microscope till the formation of adults. While observing the development of *D. magna* from juveniles to adults, the developmental period of different instars was also recorded. The body length and breadth of developmental stages of *D. magna* were measured with the help of software Magnus Pro.

3. Results and discussion

Female *D. magna* was spherical in shape and enclosed by a transparent shell like structure known as carapace. The carapace extends into head shield which is an important diagnostic feature of this species (Ebert 2005 and Haney 2010) [6, 9]. The first pair of antennae was quite small and attached to the rostrum while second pair of antennae was larger having two branches bearing long feathery bristles (Fig. 2a). Adult males can be easily distinguished from females having smaller size, larger antennae and modified hook like first appendages (Fig. 2b) as also observed by Clare (2002) [2]. Cladocerans possess a first pair of antennae or antennules,

generally with one segment and other smaller antenna with chemical sense functions. The second pair of antennae is larger and being used to swim and dorsal margin of post abdomen is sinuated (Dodson and Frey 2001) [4]. During asexual mode of reproduction, ovigerous females stored their eggs in their brood chambers lying beneath the carapace, closed by the abdominal processes (Fig. 1a) and literature reveals that these eggs developed into only females (Zaffagnini 1987) [16]. However unfavourable conditions like lack of food and population explosion may induce production of males during sexual mode of reproduction and production of resting eggs (ephippia). The resting eggs are encapsulated in a protective saddle like structure called ephippium (Fig. 1 b). These eggs may endure unfavourable conditions such as drought and variation in climatic conditions (Ebert 2005) [6]. A sexual clone can produce males and females and is capable of self-fertilization. For fertilization, males copulate with females. Fertilization is internal and takes place between molting and deposition of eggs into the ephippium (Ebert 2000) [7].

Ten female *D. magna* were taken into consideration during the present study and their body measurements were taken. The average length of female *Daphnia* measured was 2.619 ± 0.19 mm (ranging from 2.35 mm -3.01 mm) and average width was 0.694 ± 0.12 mm (ranging from 0.51 mm - 0.92 mm) (Table 1). Average egg carrying capacity i.e. number of eggs/ brood was found to be 7.00 ± 1.63 /female, with its variation ranging from 5-10 eggs/brood (Table 1, Fig. 3a) Eggs hatched into embryos within 1-2 days and embryos were found to develop in the brood chamber for approximately 3-4 days (Fig 3b). Then juveniles were released from the brood chamber through ventral flexion of the post abdomen. In case of parthenogenetic mode of reproduction, the embryo development is direct and takes place in the female incubation chamber or brood chamber. Juveniles (neonates) emerge from this chamber and are freed to the environment after completing their development inside the incubation chamber. Juveniles are similar to the appearance of the adult with a minor size (Mitchell *et al.*, 2004) [12]. On an average 4.20 ± 0.42 pre-adult instars were formed and this variability of number of pre-adult instars was found to range from 4-5 (Table 1).

Table 1: Life cycle of *Daphnia magna* observed during the present study

Ovigerous female	Length (mm)	Width (mm)	Number of eggs/brood	Number of pre-adult instars*
F1	2.49	0.60	6	4
F2	2.35	0.51	5	4
F3	2.48	0.60	7	4
F4	2.51	0.62	7	4
F5	2.50	0.61	5	4
F6	2.65	0.79	6	4
F7	2.60	0.70	7	4
F8	2.85	0.81	9	5
F9	2.75	0.78	8	4
F10	3.01	0.92	10	5
Mean \pm S.D	2.619 ± 0.19	0.694 ± 0.12	7.00 ± 1.63	4.20 ± 0.42
Range	2.35-3.01	0.51-0.92	5-10	4-5

*F1-F-10- represents the number of females taken individually for data recording

*pre-adult instar- number of instars elapsing between the time of release of individual female from brood chamber of her mother till the appearance of eggs in its own brood chamber

The life cycle of *D. magna* includes 4-5 juvenile instars/pre-adult instars till the emergence of adults. Juveniles were

exactly similar to the adults, but were smaller in size, having longer caudal spine than mature individuals. The body shape

of these juveniles was found to be thin and elongated (Fig. 3c-f) as also observed by Ranta *et al.* (1993) [14]. Time taken for the conversion of juvenile instar to adult females varied from 6-8 days and 4-6 days in males (Table 2). The average length and width of juvenile instar till the emergence of adult female varied from 0.90±0.014 to 1.77±0.02 mm and 0.097±0.004 to 0.390±0.019mm. The juvenile instars which later on became males were found to have smaller body length ranging from 0.60±0.005 mm to 0.89±0.012 mm and width varying from 0.009±0.001 mm to 0.023±0.02 mm (Table 2). Average length and width of emerged adult females was found to be 2.60±0.10 mm and 0.450±0.057mm respectively while adult

males showed average length of 1.03±0.008 mm and width of 0.140±0.019 mm, which indicated that females are bigger than males (Table 2).

During the present study it was observed that single female can produce a new clutch of eggs every 3-5 days during her life span. Ebert (2000) [7] has also observed that a female can produce a clutch of 6-10 eggs every 3-4 days until her death. Another observation made was that adult female gains their sexual maturity within 6-10 days. Overall life cycle of *D. magna* i.e. from egg to adult was completed approximately within 9-11 days and life span was found to range from 35-40 days.

Table 2: Post-embryonic developmental stages and their duration in *Daphnia magna*

Pre-adult Instar stages	Duration in days (Range)	Measurements of different stages of Female (N=5)		Pre-adult Instar stages	Duration in days (Range)	Measurements of different stages of Male (N=5)	
		Length (mm)	Width (mm)			Length (mm)	Width (mm)
1	6-8	0.90±0.014	0.097±0.004	1	4-6	0.60±0.005	0.009±0.001
2		1.09±0.018	0.225±0.007	2		0.75±0.006	0.016±0.002
3		1.35±0.015	0.316±0.006	3		0.81±0.005	0.018±0.001
4		1.40±0.019	0.388±0.017	4		0.89±0.012	0.023±0.002
5		1.77±0.020	0.390±0.019	5(Adult)		1.03±0.008	0.140±0.019
6 (Adult)		2.60±0.100	0.450±0.057				

Values are Mean ± S.D

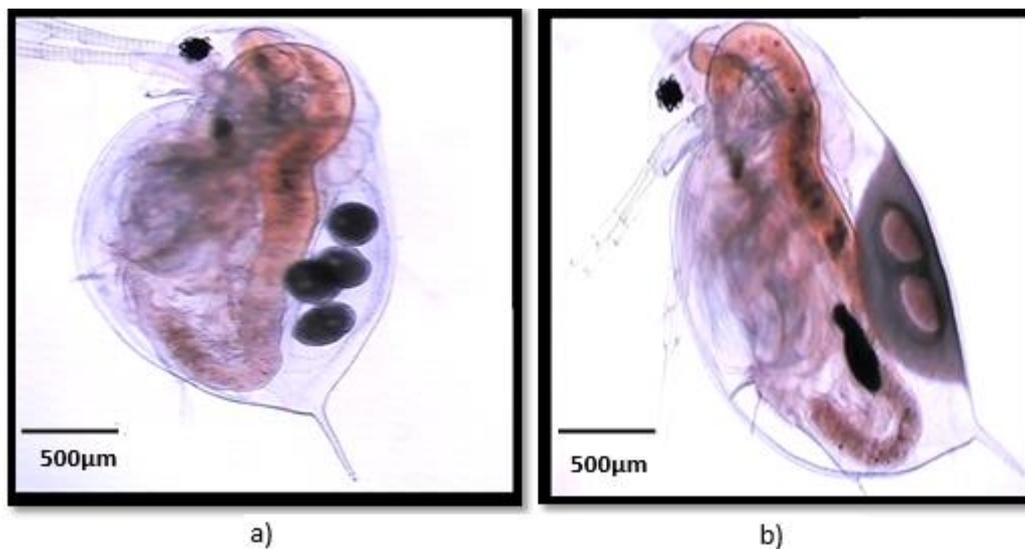


Fig 1: a) Female *Daphnia magna* carrying parthenogenetic eggs in the brood chamber (4x)
 b) Female *Daphnia magna* carrying resting eggs inside the ephippium (4x)

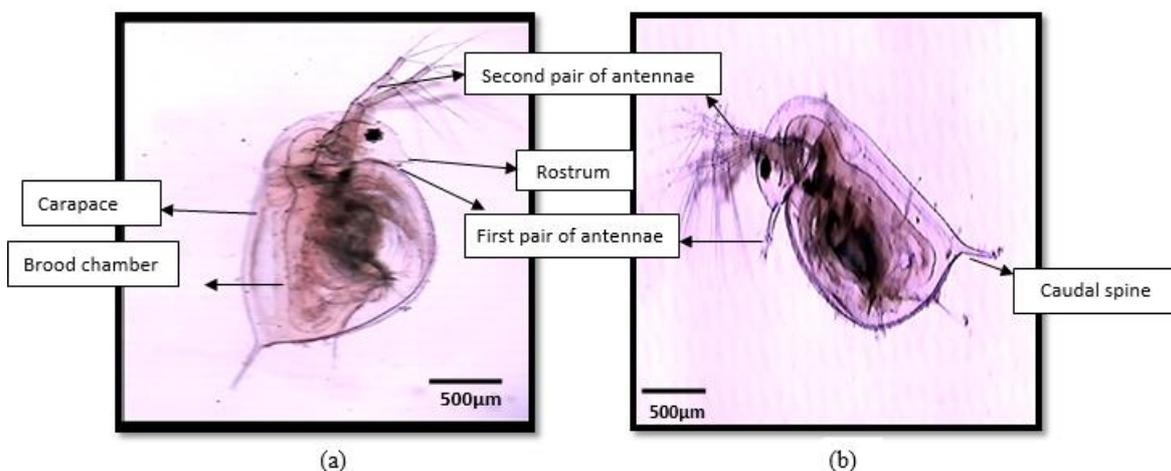


Fig 2: (a) Adult female and (b) male *Daphnia magna* (4x)

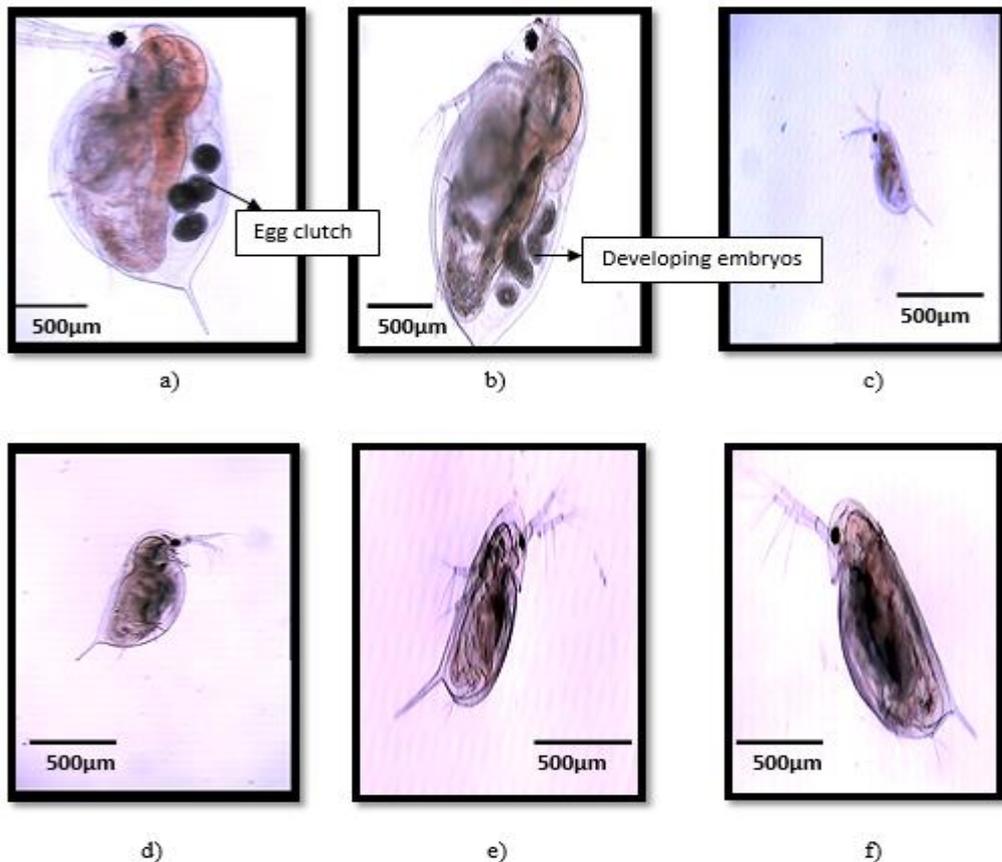


Fig 3: Developmental stages of *Daphnia magna* (4x) a) Ovigerous female with clutch of eggs inside the brood chamber b) Developing embryos inside the brood chamber c) Pre-adult instars (c-f)

4. Conclusion

Life cycle of *D. magna* was studied during the present study which showed that single ovigerous female was sufficient to start pure culture. Female *D. magna* showed its capability to produce clutch of 5-10 eggs at $22\pm 1^{\circ}\text{C}$ temperature. They attain sexual maturity within 6-10 days. They became gravid again within 4-5 days after the release of juveniles. They are easy to culture and maintain under laboratory conditions due to their short life span i.e. 30-45 days.

5. Acknowledgement

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