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Short Communication

Biology and culture of *Daphnia pulex* (Leydig) under temperate conditions in Kashmir

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Abstract

Biological studies on the growth and reproduction of *Daphnia pulex* (Leydig) under temperate conditions show that at an ambient temperature range of 19–25°C this species within a life span of 18 days has 6 pre-adult and 12 adult instars. Studies on the longevity, growth, egg production, and embryogenesis of this organism *vis-a-vis* different qualities of light and various temperature ranges have been highlighted. A technique to mass culture *Daphnia pulex* has been developed to yield a maximum density of about 9000 org./l in two weeks

Key words: *Daphnia pulex*, biology, effect of temperature and quality of light, mass culture.

1. Introduction

With recent thrust in rural aquaculture programme in the country, mass culture of selected fish-food organisms has assumed much importance, particularly in nursery management[1–2]. The success achieved in recent times to rear larval stages of some fishes and prawns under controlled conditions has been largely due to the availability of right type of food organisms[3]. Mass rearing of *Daphnia* has been attempted by many workers[4–11]. During the present study it is aimed to assess the various biological features of local *Daphnia pulex* species and to develop the methodology for its mass culture suitable for local conditions.

2. Materials and methods

Ovigerous females of *Daphnia pulex* were collected from a perennial pond near Bemina, Srinagar (lat. 34·1°N) and were initially acclimatized to the laboratory conditions. The newly hatched organisms were separated from the mothers and were used for detailed biological studies. The methods followed for the study of life history were after Murugan

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and Sivaramakrishnan[12] and Bhannot and Vass[10]. Different nutrient media of organic and inorganic nature (cow-dung, sheep manure, mustard oil cake, silk worm pupae, urea, yeast, etc.) were used during the present culture studies, ranging between 100 and 3,000 ppm in concentrations. Plastic and glass containers of 10–25 l capacity were used for laboratory experiments, while cement cisterns (1,000 l cap.) and mud ponds (1–2 m^2 area) were utilised for yard and field trials. Water analysis of each culture system was done periodically[13].

The effect of different quality of light on the general behaviour of this organism was studied. During the course of the experiments, *Daphnia* were cultured in small containers (2·5 l cap.). Transparent containers were covered completely with cellophane of blue, red and violet keeping natural sunlight as the source. However, day length was the same in all the experiments. The experimental *Daphnia* were fed with green alga – *Scenedesmus* at fixed intervals. The method has been employed by many workers[15]. The wavelengths of transmission were determined with a spectrophotometer. By following such a technique it is possible to separate out a number of bands/wavelengths each corresponding approximately to a known colour or narrow range of colours from the visible spectrum. The various growth measurements of experimental *Daphnia* were made after Buikema[14].

3. Observations

3.1. Life cycle and reproductive cycle

3.1.1. Biology of Daphnia pulex

The neonates of *Daphnia pulex* which are miniature adults have a mean length of 0·72 mm. There are six pre-adult instars (instar at initial reproduction, primiparous instar) and 12 adult instars. The body length of the first adult instar (7th instar) is 1·66 mm. An average maximum length of 2·20 mm is attained during 17th and 18th instar in a mean life span of 18 days (Table I) at a temperature range of 19–25°C. Since various experiments were performed during different seasons, it was observed that temperature has a profound influence on the biology of the animal (Table II).

3.1.2. Hatching of ephippial eggs

To have a sustained supply of inoculum of this species, attempts were made to hatch the resting eggs (ephippial) of *D. pulex* under laboratory conditions. The ephippial eggs, after dessication and freezing for 5–8 days, were subjected to high temperature treatment (range 30–35°C) for about 4 h. Thereafter, the eggs were kept for hatching at a temperature range of 10–15°C and by this method 40–60% of the eggs could be hatched under laboratory conditions.

3.1.3. Effect of different quality of light on the growth

Earlier studies suggest that intensity and quality of different light affect the growth of Cladocerns[16–19], Ostracodes[20] as well as other organisms[21–22]. During the present

Table I
Mean length, number of eggs per brood and cumulative frequency of egg production in each instar of *Daphnia pulex* (19–25°C)

Instar no.	Mean length (mm)	No of eggs per brood (mean)	Cumulative frequency of egg production per instar (mean)
1	0.72	–	–
2	0.82	–	–
3	1.14	–	–
4	1.22	–	–
5	1.28	–	–
6	1.58	–	–
7	1.68	5.00	5.00
8	1.78	–	5.00
9	1.82	7.65	12.65
10	1.86	10.71	23.36
11	1.90	–	23.36
12	1.91	12.00	35.36
13	1.94	–	35.36
14	1.98	15.15	50.51
15	2.04	6.00	56.51
16	2.16	8.00	64.51
17	2.20	9.00	73.51
18	2.20	5.16	78.67

investigations individuals of *D. pulex* were raised under different lights *viz.*, red, green, blue, and violet and total darkness in order to study their effect on various biological features which would in turn have some impact on their mass culture. The study revealed that on the whole, animals showed positive response towards blue light (Table III).

3.2. Culture experiments

3.2.1. Under laboratory conditions

The data on the growth of *D. pulex* reared under different experimental conditions were studied. Comparing the growth pattern of this animal in different media *viz.*, pond water, lake water and stream water, but maintaining the diet *Scenedesmus* in all the sets, the study conducted for two weeks revealed that pond water yielded 340 times more abundance in the population as compared to that of the lake water (184 times) and stream water (7 times). The average variations in chemical conditions of the media are tabulated in Table IV.

The study implies that apart from feeding, the nutrient status of the medium has a profound influence on the growth of *Daphnia pulex*. It was also observed that by enriching the medium with different kinds and levels of organic manure, growth of

Table II
Effect of different temperatures on the biology of *Daphnia pulex*

Temperature range (°C)	Size at first instar (mm)	Pre-adult instars	Size at first maturity (mm)	Adult instars	Size at last instar (mm)	Total life span (days)	Average no. of eggs/ brood	Embryo-genetic period (days)	Total no. of eggs per individual
5.0–11.0	0.58	30	1.64	31	2.47	61	13.74	6–8	83
10.0–18.0	0.56	11	1.51	23	2.34	34	8.16	4–5	91
19.0–25.0	0.72	6	1.66	12	2.20	18	7.67	1–2	79

Table III
Effect of light quality on growth, molting, reproduction and survival of individually cultured *Daphnia pulex*

Light quality	Approx. spectral range (millimicrons)	Length on first day (mm)		Length prior to release of first brood (mm)		Days prior to release of first brood		Length after 15th day (mm)		Molting rate per animal per day		No of broods/ adult		No. of young/ animal		Survival in days	
		N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean
Red	640–700	10	0.74	10	1.56	8	6.5	8	2.14	8	0.57	8	7 2	8	76 11	7	19.5
Green	500–580	10	0.72	10	1.54	9	7.4	9	2.05	8	0.61	8	6.4	8	74.16	7	16.6
Blue	400–465	10	0.74	10	1.60	9	5.8	9	2.14	9	0.56	9	8.4	8	91.20	7	21.3
Violet	390–430	10	0.71	10	1.64	8	7.6	8	2.09	8	0.62	8	6.4	8	73.17	7	18.4
Darkness	nil	10	0.71	10	1.64	8	7.5	8	2.26	7	0.64	7	6.2	7	66.84	6	18.1

N =Number of organisms experimented.

Table IV
Chemistry of various water types under different experimental conditions

Parameters	Pond water	Lake water	Stream water
pH (units)	7.6–8.2	7.2–8.0	7.0–7.4
Total alkalinity (ppm)	80.0–120.0	80 0–110 0	45.0–65 0
Specific-conductance (micromhos/25°C)	260.0–440.0	200 0–380 0	80.0–120.0

Table V
Effect of different nutrient media (at a concentration of 2g/l in pond water) on the culture of *Daphnia pulex* in laboratory conditions

Sl. no.	Nutrient media	Initial stocking (org./l)	Numerical abundance			Magnitude of increase
			7th day (org./l)	14th day (org./l)	21st day (org /l)	
1.	Cow-dung extract	50	735	1,695	3,176	64 times
2.	Sheep manure	50	510	1,172	2,160	43
3.	Mustard oil cake	50	248	680	1,310	26
4.	Urea	50	338	867	1,527	30
5.	Yeast	50	276	732	1,433	28
6.	Silk worm pupae	50	310	810	1,572	31

Table VI
Effect of various concentrations of cow-dung extract used on the abundance of *D. pulex* in laboratory conditions

Sl. no.	Concentration of cow-dung extract (g/l)	Initial stocking (org./l)	Numerical abundance by the 7th day (org./l)	Increase or decrease
1.	2.00	100	1,600	+ 16 times
2.	4.00	100	1,276	+ 12.7
3.	8.00	100	510	+ 5.0
4.	12.00	100	76	– 0.24

(org./l = Organisms per litre)

Daphnia could be increased within a specific time period (Tables V and VI). The study indicates that best results were obtained with cow-dung extract at mild concentrations while the higher concentrations produced a retarding effect.

3.2.2. Under yard and field conditions

Mass culture of *Daphnia pulex* was successfully achieved under yard conditions in small cemented tanks (capacity 1,000 l). Under experimental studies, one tank was provided with a substrate of soil and dry cow dung as medium and allowed to fertilize for 3–4 days before any inoculum could be introduced. In another tank no soil or nutrient medium was added. With an initial inoculum of 60 ind./l in both the tanks, after three weeks rearing period a peak population of 8,000 ind./l was recorded in the treated tank and 4,300 ind./l in untreated control tank at a temperature range of 20–25°C. Both the tanks were supplemented with cow-dung extract @ 2,000 ppm after every third day to maintain the optimum level of nutrition.

Another trial was conducted in small mud ponds to culture *Daphnia pulex* and good results were achieved comparable to yard experiments. The pond was initially fertilized with cow dung @ 2 kg/m^2 and after complete fertilization of nutrients in about four days period, the inoculum of *Daphnia* was introduced @ 100 ind./l. After rearing for about two weeks the organism registered a peak population of 9000 ind./l at a temperature range of 18–25°C. During the period nutrients were added every fifth day.

During the present investigations a significant depression in the population was noted in case the cultures of *D. pulex* were maintained for longer periods at higher densities. This population depression has been attributed to infection by ciliates and oxygen depletion in open yard culture systems. Such observations have also been recorded by Fox[23] and Green[24]. These factors directly affect the egg output, growth, rate of maturation and increase in the population of males.

In order to overcome the population depression, during the present studies a yard experimental trial was conducted in which *Daphnia* population was periodically harvested and by providing constant aeration sustained population was maintained for 3–4 months. By this method the overall biomass could be increased by 25%.

4. Discussion

The variation in instar numbers has been attributed to either heredity or the culture medium[25]. But during the present study it was observed that besides these two factors, temperature has a significant effect on the biology of the organism. At an optimum temperature range of 19–25°C, *Daphnia* undergoes 18 instars (6 pre-adult and 12 adult instars) within an average life span of 18 days. At a temperature range of 5–11°C the animal has 30 pre-adult and 31 adult instars and attains a maximum size of 2·47 mm. However, at 10–18°C it exhibits 11 pre-adult and 33 adult instars, with a maximum size of 2.31 mm. Similar variations in instar numbers have also been reported on different microcrustacea both from tropical and temperate waters by many workers[26–28].

The present study also revealed a significant relationship between embryonic development and temperature, which was 1·5 days at 22°C, 4·5 days at 14°C and 7·0 days at 8°C. Gross and Bunting[11] also recorded similar relationship in *D. pulex* and *D. magna*.

Pre-adult length increment as a percentage of the initial length of the organism has been studied in both tropical and temperate cladocerns. In some temperate cladocerns, Green[24] reported maximum growth increment during pre-adult instar in *Daphnia thomsoni*, *D. magna*, *D. atkinsoni* and *D. pulex*. In this species, the observed growth increment is 32% at the first instar and by the 6th pre-adult the growth is low, 28%. In *Simocephalus acutirostratus*, Murugan and Sivaramakrishnan[12] reported that the species attains maximum growth increment in the third pre-adult instar (84%) and it is the highest growth increment recorded for a temperate/tropical species. *Moina micrura* shows minimum growth increment during the pre-adult phase and recovers growth only during the adult instars[29].

The variation in egg production in cladocerns has been attributed to many factors *viz.* amount of food available[25,30] genetic make-up of the animal[31] and temperature of the culture medium[32] which has a direct impact on the production of progeny per brood and rapid embryogenesis. *Daphnia pulex* has a capacity to produce maximum 31 eggs/brood at lower temperatures (5–10°C) at a time, while at 10–18°C it produces 25 eggs/brood compared to 15 eggs/brood at 19–25°C. The mean number of eggs per brood in relation to total instar number of *Daphnia pulex* is compared with that of other cladocerns. The present species has the highest number of 4·0 eggs per brood in a life span of 18 days (19–25°C) which decreases to 1·0 in a life span of 61 days at a temperature of 5–10°C. On the other hand, *D. senegal* is reported to produce lesser number (3·5 eggs at 9th instar[12]; in the same species, Venkataraman and Krishnaswamy[2] reported a maximum 6 eggs per brood (28–30°C) in a life-span of 56 days. Murugan and Sivaramakrishnan[12] recorded 27 eggs per brood in *S. acutirostratus*.

The effect of different quality of light on the biology of *Daphnia pulex* in the present experiments revealed that mean body length of 15-day population was longer (1·60 mm) in blue light compared to other types of lights, but slightly lower than the individuals raised in total darkness (1·64 mm). It was also noted that under blue light the life span of the individual was high (21·3 days) with a marginal increase in total production (91·2 ind./organism). Similarly, molting rate expressed as molts/day/animal was higher in total darkness (0·64 molts/day/animal) but lower (0·56 molts/day/animal) under blue light conditions. The growth behaviour *vis-a-vis* egg production, life-span, etc., of *Daphnia pulex* under the blue light was significantly better compared to other light conditions.

5. Conclusion

The present investigation revealed that the overall population and biomass production of this organism could be maintained at an optimum level and even increased many folds by

thinning the culture periodically, and coupled with proper aeration, the culture could be kept at a high reproductive capacity for as long as 3–4 months. This culture technology would help in a big way for nursery rearing of endemic Schizothoracid fishery under temperate conditions.

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References

1 FERNANDO, C.H. Reservoir and lakes of Southeast Asia (oriental region) O. *Lakes Reservoir Taub.*, 1984, **17**, 411–448.

2. VENKATARAMAN, K. AND KRISHNASWAMY, S. Influence of modified Banta's medium and pond water on the longevity, egg production and body size of *Daphnia similis* and *D. cephalata* (Cladocera) under laboratory conditions, *Proc. Indian. Acad. Sci. (Anim. Sci.)*, 1986, **95**, 247–254.

3. LASKER, R., FEDER, H.A., AND THEILACKER, G.H. Feeding growth and survival of *Engraulus mindax* larvae reared in laboratory, *Mar. Biol*, 1970, **5**, 345–353.

4. BANTA, A.M. A convenient culture medium for daphnids, *Science*, 1921, **53**, 557.

5. BOGATOVA, I.R. Experiment in the cultivation of the plankton fodder for the Sturgeon juveniles, *Trans. Savator, Section Kaspian Br. VNIRO*, 1951, **1**, 148–162.

6. BOGATOVA, I.R. AND AIKEROV, N.K. Experiment in commercial cultivation of daphnidae, *Rybn. Khoz.*, 1958, **12**, 21–26.

7. MURPHY, R.G. A general method for the monoxenic cultivation of the Daphnidae, *Biol. Bull.*, 1970, **139**, 321–332.

8. HARVEY, A.M. Semicontinuous culture technique of *Daphnia pulex*, *J. Appl. Ecol.*, 1972, **24**, 84–97.

9. IVLEVA, I.V. Mass cultivation of invertebrates, biology and methods, *Jerusalem Stand. Geogr.*, 1973, 193.

10. BHANNOT, K.K. AND VASS, K.K. Mass rearing of *Daphnia carinata* King, in the field, *J. Inland Fish. Soc. India*, 1976, **8**, 145–148.

11. GROSS, L.B. AND BUNTING, D.L. *Daphnia* development and reproduction responses to temperature, *J. Thermal Biol.*, 1983, **8**, 375–380.

12. MURUGAN, N. AND SIVARAMAKRISHNAN, K. G. The biology of *Simocephalus acutirostratus* King, (Cladocera): Laboratory studies of life cycle, instar duration, egg production, growth and stages in embryonic development, *Hydrobiologia*, 1973, **50**, 75–80.

13. *Standard methods for the examination of waste and water*, APHA, New York, 1975.

14. BUIKEMA, A.J. — Some effects of light on the growth, molting, reproduction and survival of the Cladocern, *Daphnia pulex*, *Hydrobiologia*, 1973, **41**, 391–418.

15. MEYER, B.S. ANDERSON, D.B AND BOHNING, R.H — *Introduction to plant physiology*, D. Van Nostrand Co , New York, 1960, 530.

16. SCHULZ, G.C. — Ober die bedeutung des lichtes in lebern neiderer krebes, *Z. Vergl. Physiol.*, 1928, 488–552.

17. EDLEN, A. — Wachstum and milica bei *Daphnia magna*, *Lund*, 1943, 1–125.

18. BUIKEMA, A.J. — Effects of varying wavelengths, intensities, and polarised light on population dynamics and ephippial production of *Daphnia pulex* Leydig, *Crustaceana*, 1968, **14**, 46–55.

19. HAVEL, J.E. AND DODSON, S.I. — Environmental cues for cyclomorphosis in *Daphnia retrocurva*, *Freshwat. Biol* , 1985, **14**, 469–478.

20. KLUGH, A.B. — The effect of ultraviolet component of sunlight on certain aquatic organisms, *Can. J Res.*, 1929, **1**, 100–109.

21. STEPHENS, G.C — Induction of molting in the cryfish, *Cambarus*, by modification of daily photoperiod, *Biol. Bull.*, 1955, **108**, 235–241.

22. RAO, C.N. SHAKUNTALA, K. AND REDDY, S.R. — Regulations of ovigevicity in a freshwater prawn, *Int. J. Invertebrate Reproduction Dev.*, 1985, **8**, 185–188.

23. FOX, H.M. — The haemoglobin of *Daphnia*, *Proc. R. Soc. B.*, 1948, **13**, 195–212.

24. GREEN, J. — Studies on a population of *Daphnia magna*, *J. Anim. Ecol.*, 1955, **24**, 84–97.

25. ANDERSON, D.G. AND JENKINS, J.C. — A time study of events in the life span of *Daphnia magna*, *Biol Bull.*, 1942, **83**, 200–272.

26. — *Biology and culture of temperate fish-food organisms*, Central Inland Fish. Res. Inst. Report. Memo., 1982, p. 25.

27. NAVANEETHAKRISHNAN, P. AND MICHAEL, R.G. — Egg production and growth in *Daphnia carinata* King, *Proc. Indian Acad. Sci. (B)*, 1971, **73**, 117–123.

28. VENKATARAMAN, K. — Field and laboratory studies in *Daphnia carinata* from a seasonal tropical pond, *Hydrobiologia*, 1981, **78**, 221–225.

29. MURUGAN, N. — The biology of *Ceriodaphnia cornuta* Sars (Cladocera), *J. Inland Fish. Soc. India*, 1975, **7**, 80–87

30. DUNHAM, H.H. — Abundant feeding followed by restricted feeding and longevity in *Daphnia*, *Physiol. Zool.*, 1938, **11**, 399–407.

31. BANTA, A M. AND WOOD, T.R. — *General studies in sexual reproduction* (in Banta, 1939), 1939, 131–181.

32. MCARTHUR, L.W. AND BAILLIE, W.H.T. — Metabolic activity and duration of life influence of temperature on longevity in *D. magna*, *J. Expl Zool.*, 1929, **53**, 221–242.