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Effect of temperature on mass culture of three species of zooplankton, *Brachionus plicatilis, Ceriodaphnia reticulata* and *Apocyclops dengizicus*

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Abstract
In larval rearing of commercially between important species appropriate live feed is necessary due to their small size, small gap, fragile nature, not fully developed receptors and digestive system. It is well established that the live food organisms meet all the necessary criteria for the small larvae of all cultivable fishes. Considering the importance of zooplankton as live feed, present study reports influence of temperature (26 ± 1 ºC and 31±1 ºC) on the population density in the mass culture of the rotifer *Brachionus plicatilis*, cladoceran *Ceriodaphnia reticulata* and Cyclopoida copepod *Apocyclops dengizicus* in chicken manure fertilized medium. At 26±1 ºC, all the three species viz. *B. plicatilis*, *C. reticulata* and *A. dengizicus* showed progressive increase in density and peaked during the third week of experimentation and their densities were 30833±1644 No./L, 8466±907 No./L and 14900±2981 No./L, respectively. At 31±1 ºC, density of all the three species of zooplankton peaked in the second week of culture period and their densities were 9133±1778 no. /L, 1200±458 No. /L and 6000±1228 No. /L, respectively. One way ANOVA showed significant differences in their density 0.5 level of confidence at low and high temperatures.

Keywords: Aquaculture, live feed, zooplankton, temperature, mass culture

1. Introduction
Production of quality seeds with a high survival rate is important for the economy of the fish culture industry. In hatcheries, growth and survival of developmental stages of many finfish and shellfish depends on providing nutrient enriched suitable live feed. Zooplankton play a vital role as natural food for fishes, particularly from endo-exogenous to exclusively exogenous feeding stages. Successful mass culture of zooplankton such as rotifer [1], cyclopoid copepod [2] and cladoceran [3] were reported using algae and animal wastes. For mass culture, knowledge on the aspects of cultivable species such as life span, food and feeding habits and reproductive potential are essential. Physico-chemical parameters in general and temperature in particular influences the life cycle and reproduction of many zooplanktonic species. Influence of temperature on the fecundity of zooplankton such as the rotifer *Brachionus plicatilis* [4] , cladoceran *Daphnia* [5, 6], copepod *Paracalanus parvus* [7] and *Thermocyclops* crasses and *Eudiaptomus drieschi* [8] were reported. Ideal physicochemical parameters prevalent in the Indian subcontinent support rich diversity of zooplankton in fresh water, estuarine and marine waters. These organisms constitute a natural feed for the commercially important adult fish and their larvae. In spite of having ideal climatic condition and high biodiversity of zooplankton, protocol for mass culture of zooplankton species is yet to be established. Due to the non-availability of indigenous live feed consistent hatchery seed production of edible and ornamental fish is not achieved. In order to mass culture important zooplankton species, intensive research should be carried out on their cultural aspects such as food and feeding habits, reproduction and influence of physico-chemical parameters on their population dynamics. Establishment of culture procedure for their production round the year will promote hatchery seed production of fish. In the present article, influence of temperature on the population density of mass culture of the rotifer *Brachionus plicatilis*, cladoceran *Ceriodaphnia reticulata* and cyclopoid copepod *Apocyclops dengizicus* in chicken manure fertilized medium is reported.

2. Material and Methods

For the present study, the rotifer (*B. plicatilis*) and cyclopoid copepod (*A. dengizicus*) were collected from Adyar estuary and the cladoceran (*C. reticulata*) from Padi freshwater pond Chennai (Figures 1, 2 and 3). They were sieved through different sized mesh filters (100-300 µm) and were separated using stereoscopic binocular dissection microscope.

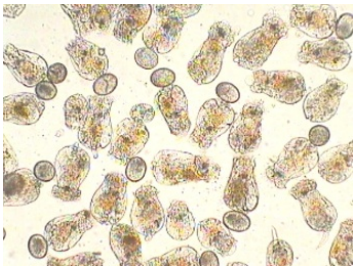

Fig 1: *Brachionus plicatilis*

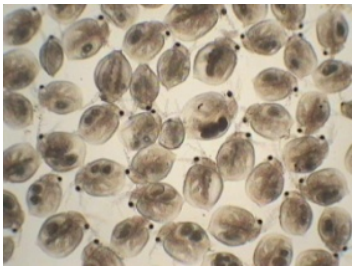

Fig 2: *Ceriodaphnia reticulate*

Fig 3: *Apocyclops dengizicus*

Chicken manure was collected from a local broiler chicken shop and dried for 5 days to remove the moisture and stored in a plastic container for fertilizing zooplankton culture medium. Chicken manure was micronized by grinding and required quantity was dissolved in water to get suspensions of stock solution and was used to fertilize culture medium for mass culture of zooplankton. The three different zooplankton species were cultured in 500 liter tanks for 35 days. Each culture tank was filled with 100 liters of filtered water of 17 to 25 ppt salinity for *B. plicatilis* and *A. dengizicus* and 2 to 4 ppt salinity for *C. reticulata.* The medium was fertilized with stock solution of chicken manure at a rate of 500 ppm for all three species. Mass culture experiments were conducted in triplicate for each species of zooplankton. The medium was continuously aerated to maintain DO of 2.0 to 4.0 mg/L and mixed micro algae containing species of *Chlorella, Ankistrodesmus, Scenedesmus, Spirogyra and Nevicula* were introduced 24 h after preparation of culture media. PH of the culture media was maintained between 7.8 and 8.3. For all the species inoculum was introduced in each tank at the rate 100 ind./L. The culture media were fertilized with chicken manure on alternate days during culture period and every week 30% of water replenished. The culture tanks were covered with mosquito net to avoid infiltration of mosquito larvae in the culture. Physicochemical parameters of the culture media were recorded every week. Mass culture experiments of these species were conducted during winter and summer by maintaining water temperature at 26 ± 1 ºC and at 31±1 ºC, respectively to quantify population density of cultured species, culture medium was thoroughly mixed and 1 L of sample was drawn from the culture medium. Subsamples of 100 ml and then 10 ml were drawn from these samples. All stages of zooplankton were counted under a binocular microscope using Sedge-wick Rafter cell. Triplicate of each sample was analyzed to determine the population of *B. plicatilis*, *C. reticulata* and *A. dengizicus*. Experimental data was statistically analyzed for ANOVA using SPSS software ver. 11.5.

3. Results

The Physical and chemical parameters of water samples of culture medium of zooplankton during culture at 26±1 ºC and 31±1 ºC temperatures are presented in Table 1. Weekly analyses of these parameters at lower temperature showed less variance with regard to pH, DO, Nitrite and Phosphates. Nitrates and Ammonia showed increased levels during the latter part of the experiment. At higher temperature, the water temperature, pH, Salinity, Dissolved Oxygen and nutrients in all the culture media did not show any marked variation.

Table 1: Physicochemical parameters during mass culture of zooplankton at low and high temperatures

Env. Parameters	*B. plicatilis*	*C. reticulata*	*A. dengizicus*
Low Temperature (26±1 °C)			
Atm temp.(°C)	27-28	27-28	27-28
Water temp. (°C)	24-27	24-26	24-26
pH	7.8-8.2	7.8-8.1	8-8.3
Salinity (ppt)	17-21	4-6	17-20
DO(mg/L)	2.36-3.81	2.12-3.93	2.48-3.93
Nitrite (mg/L)	0.5-5	0.04-0.12	0.09-0.75
Nitrate(mg/L)	5-120	1-80	2-90
Ammonia(mg/L)	11-21	7-15	10-15
Phosphate(mg/L)	5-8	2-5	3-8
High Temperature (31±1 °C)			
Atm temp.(°C)	32-34	32-34	32-34
Water temp. (°C)	29-32	29-32	29-32
pH	8-8.3	7.8-8.1	7.9-8.3
Salinity (ppt)	15-22	2-5	17-25
DO(mg/L)	2.12-3.93	2.48-3.84	2.12-3.81
Nitrite (mg/L)	0.05-0.09	0.04-0.09	0.03-0.08
Nitrate(mg/L)	1-4	1-20	1-10
Ammonia(mg/L)	5-9	3-6	6-8
Phosphate(mg/L)	4-6	1-4	2-6

At 26±1 ºC temperature, all the three species viz. *B. plicatilis*, *C. reticulata* and *A. dengizicus* showed progressive increase in density from the beginning of the culture and peaked during the third week of experimentation and their densities at the end of third week were 30833±1644 No./L, 8466±907 No./L and 14900±2981 No./L, respectively. During the fourth week *B. plicatilis* sustained the population density while during the fifth week population of all the three species declined to low levels. At 31±1 ºC, remarkable difference was recorded in the density of all the three species of zooplankton compared to the culture at 26±1 ºC. The population structure in the different weeks of the culture period at low and high temperatures also varied considerably. During first week of culture period under high temperature only 50% of density of rotifers and cyclopoid copepod was recorded compared to the low temperature experimentation period. At 31±1 ºC temperature, density of all the three species of zooplankton peaked in the second week of culture period and their densities at the end of second week were 9133±1778 No./L, 1200±458 No./L and 6000±1228 No./L, respectively. From third week onwards population density of these species reduced significantly (Table 2). One way ANOVA showed significant difference in their density at 0.5 level of confidence at low temperature (rotifer: df = 17, F = 65.215, p = 0.00; cladoceran: df = 17, F = 93.216, p = 0.00; cyclopoid copepods: df = 17, F = 38.404, p = 0.00) and high temperature (rotifer: df = 17, F = 36.335, p = 0.00; cladoceran: df = 17, F = 10.433, p = 0.00; cyclopoid copepods: df = 17, F = 34.570, p = 0.00).

Table 2: Density of zooplankton during low and high temperature culture

	B. plicatilis	*C. reticulata*	*A. dengizicus*
Low temperature (26±1°C)			
1 Week	4766.66±351.18	266.66±152.75	1566.66±585.94
2 Week	13333.33±577.35	2333.33±577.35	9666.66±2081
3 Week	30833.33±1644.18	8466.66±907.37	14900±2981.61
4 Week	28833.33±5499.39	5566.66±321.45	7466.66±1059.87
5 Week	12833.33±3073	2766.66±862.16	3166.66±208.16
High temperature (31±1 °C)			
1 Week	2066.66±351.18	733.33±152.75	800±100
2 Week	9133.33±1778.57	1200±458.25	6000±1228.82
3 Week	6000±1228.82	533.33±57.73	1400±300
4 Week	3633.33±611.01	633.33±152.75	600±173.2
5 Week	2100±264.57	233.33±57.73	2733.33±503.32

The zooplankton cultured at two different temperature ranges (26±1 ºC and 31±1 ºC) showed differences in their body size. *B. plicatilis* and *A. dengizicus* cultured at lower temperature were larger in size than those cultured at higher temperature range. The length and width of three zooplankton species are presented in Table 3. The egg diameter of *B. plicatilis* cultured at 26±1 ºC and 31±1 ºC was 85±14 µm and 80±09 µm, respectively while that of *A. dengizicus* it was 84±12 µm and 78±09 µm, respectively. The length and width of adult *C. reticulata* cultured at 26±1 ºC and 31±1 ºC did not show variation.

Table 3: Body dimensions of zooplankton cultured at low and high temperature culture

Zooplankton species	Temperature of culture medium 26±1 ºC		Temperature of culture medium 31±1 °C	
	Length (µm)	Width (µm)	Length (µm)	Width (µm)
B. plicatilis adult	205±24	124±16	174±10	113±25
C. reticulata adult	485±74	365±75	523±47	444±85
C. reticulata neonate	160±33	128±24	166±23	133 ±21
A. dengizicus male	736±23	184±11	688±29	180±8
A. dengizicus female	864±42	285±9	827±33	256±16
A. dengizicus nauplius	253±18	128±9	247±42	117±12
A. dengizicus copepodid	544±30	178±15	558±74	156±38

4. Discussion

The results of culture of zooplankton under two different temperature ranges showed variation pertaining to the duration of development, body size and population density. With regards to the development period of zooplankton, increase in temperature accelerates embryonic and post embryonic development which led to the faster attainment of peak density of all the three species of zooplankton at the end of second week of culture in higher temperature range compared to the attainment of peak density of all the three species of zooplankton at the end of third week under lower temperature. The egg production and hatching rate are generally increased with increasing temperature up to a temperature threshold, after which decline begins. Earlier laboratory studies showed that temperature affects the egg production and the survival rate of zooplankton [9, 6]. Although, the egg diameter of *B. plicatilis* and *A. dengizicus* showed slightly higher size in low temperature conditions than those cultured in high temperature, there no statistical significance at 0.5 level. Body size and temperature are the two most important variables affecting nearly all biological rates and times. The relationship of size and temperature to development is of particular interest because during ontogeny size changes and temperature often varies [10]. Statistically significant higher body length and width of adults of *B. plicatilis* and *A. dengizicus* is recorded in low temperature culture than in high temperature culture. However, such variation is not evident in the case of the length and width of the nauplii and copepodids of *A. dengizicus* cultured in two different temperature conditions. In the case of adults and neonates of *C. reticulata* marked difference is not recorded in their length and weight at two different culture temperature. Many investigations reported that one of the reasons of the reduced body size of zooplankton might be the increase in temperature due to global warming [11-13]. Daufresne *et al.* [11] in a mesocosm experiment on the influence of temperature on marine copepods reported that they became smaller with increasing water temperature. Further, these authors opined that an increase in temperature may result in a decrease in mean body size of aquatic ectothermic animals at the community, population or individual level. Influence of temperature at the community level might increase the proportional contribution of small-sized species, at the population level a shift in size, structure toward a higher proportion of smaller individuals and at the individual level a decrease in individual size at age or stage [14, 11]. In addition to temperature, average body size in a population will also be affected by other environmental variables such as food availability, competition, predation, or parasitism [15, 16]. In addition to temperature, different environmental variables might also influence the body size. The weekly analysis of densities of zooplankton species cultured under two different temperature conditions indicated different population dynamics of these species. In the culture at low temperature, at the end of first week higher population density of *B. plicatilis* and *A. dengizicus* was recorded than the *C. reticulata*. This might be due to the higher parthenogenetic proliferation rate of rotifers than the Cladocerans, while higher fecundity in the case of cyclopoid copepod might be the reason for their higher population density than the cladoceran. However, in the culture at high temperature, at the end of 1st week higher population density of *B. plicatilis* was recorded than the *A. dengizicus* and *C. reticulata*. This might be consequence of differential influence of temperature on the reproductive rate of these zooplankton species. At the end of second week of culture substantial increase in the population density of all the three species of zooplankton was observed. The attainment of peak density during the second week and third week in high temperature and low temperature culture, respectively obviously indicate accelerative influence of temperature on the development of these zooplankton species. Nevertheless, significant difference in the density of all the three species cultured at low and high temperature indicated that temperature above 30 ºC is unsuitable for the mass culture of these species, while temperature range from 25 ºC to 28 ºC appeared to be suitable for the mass culture of *B. plicatilis*, *C. reticulata* and *A. dengizicus*. The density of all the three species declined from third week in high temperature culture and from fourth week in the case of low temperature culture. The optimum water temperature reported for culture of rotifer is 25-30 °C [17], for cyclopoid copepod 25-30 °C [2] and for Cladocerans is 25-29 °C [3]. However, different optimal temperatures have been reported for the culture of different rotifer strains. *B. rotundiformis* were most productive at high temperatures 30.8 ºC. While *B. plicatilis* were most productive at lower temperatures 25.8 ºC [18-20]. The optimum water temperature for rotifer growth was also reported to be 22 to 30 °C [21].

5. Conclusion

The present study indicated that the chicken manure form an ideal fertilizer, which along with mixed algae promote mass culture of the rotifer, cladoceran and copepods species. From second week of culture zooplankton can be harvested and used in larval rearing. Temperature exerts definite influence on the density and population structure of all the three species of zooplankton species experimented and temperature of 26 to 28 °C may be ideal for the culture of these species.

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