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THE POPULATION STRUCTURE AND THE EFFECT OF pH ON GROWTH CHARACTERISTICS OF *BRACHIONUS CALYCIFLORUS AMPHICEROS* (EHRENBURG) GROWN ON FRESHWATER *CHLORELLA* SP.

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Abstract: A freshwater rotifer, *Brachionus calyciflorus amphiceros* (Ehrenberg,1838) isolated from a fish tank in Colombo was cultured using a freshwater *Chlorella* sp. isolated from the same environment as food. The population of *B. calyciflorus* was studied for their various body and egg sizes. The effect of pH on population density, growth and the rate of appearance of mictic females were also studied under similar conditions. The results indicate that the rotifer population studied consisted of 3 size groups; mictic male producing females (239.56 ± 8.85 µm, 164.26 ± 6.54 µm length and width) produced eggs of 80.45 ± 8.78 µm length and eggs hatched to give rise to males of 88.68 ± 9.11 µm length, mictic females (304.09 ± 1.68, 203.62 ± 5.21 µm length and width) produced resting eggs of 163.79 ± 4.64 µm length. Amictic females (276.44 ± 14.99, 183.06 ± 3.61 µm length and width) produced eggs of 122.63 ± 4.87 µm length. There were no significant effects between pH ranges of 5 to 8 on the population density and growth rates. The rate of appearance of mictic females was significantly affected by pH and its lowest effect was at pH 7.0. Hatching time of resting eggs increased when stored (5 - 15 months) while hatchibility decreased from 66.66% to 40.10% during this period.

Keywords: *Brochionus calyciflorus*, rotifer.

INTRODUCTION

The main use of rotifers in aquaculture has been as a larval food, with the marine rotifer, *Brachionus plicatilis* playing the most important role .[1,2] The literature on biology and mass culture of this species has been extensively reviewed [1,3,4] and the technology of rearing has been well established.[5]

Sri Lanka is an emerging ornamental fish exporter. In the international ornamental fish trade, mass production of larvae and juveniles of required ornamental fish is necessary to achieve the export goals. The rotifer, *Brachionus calyciflorus* is commonly found in freshwater bodies in Sri Lanka and could contribute immensely as a larval food. Recently *B. calyciflorus* and *B. ruben* have become the most commonly cultured rotifers in freshwater aquaculture.[1] However, information on biology and mass production culture techniques of these species are scanty and critical culture conditions have not been well established.

This work, therefore was undertaken to investigate some aspects of biology and the effects of pH on some population growth characteristics of *Brachionus calyciflorus* isolated from freshwater fish pond, under laboratory conditions.

METHODS AND MATERIALS

Brachionus calyciflorus amphiceros (Ehrenberg,1838) used in this work was isolated from a freshwater fish tank at the aquaculture experimental unit of Ceylon Grain Elevators Limited, Colombo. The freshwater *Chlorella* sp. used as food for the rotifers was also isolated from the same environment. Guillard F medium[6] was employed for the initial isolation and subsequent culture of the alga on plates, tubes and 20 litre glass carboys. Rotifers were maintained on *Chlorella* in aerated 250 ml conical flasks, at 25.0 ± 0.5°C (algae room) under constant illumination (white fluorescent lamp, 2000 Lux).

Morphometric characteristics of eggs and rotifers: Two replicate *Chlorella* cultures of 400 ml (4.8×10^6 cells ml^{-1}) were inoculated with 20 ml of rotifer (13 ind.ml^{-1}) and placed under constant illumination in an algae room at 25.0 ± 0.5°C with aeration at 0.6 l min^{-1}. On the 4th day , after mixing the culture by stirring, samples containing about 500 individuals were collected and examined microscopically. Specimens were sorted into 4 types; resting egg carrying females, females carrying medium size eggs, females carrying small size eggs and males. After preliminary observations, the animals were killed quickly by adding 10% formaldehyde and about 280 randomly selected rotifers were measured for their length (from tip of posterior median spine to tip of anterior median spine) and width. A similar number of eggs was also measured for their length. Another set of measurements, the width of animals and the length of eggs while the eggs were still attached to the animal, was taken to ascertain the sizes of eggs carried by rotifers of different sizes. Frequency distribution graphs were plotted using length and width of animals and length of eggs. The mean length and width of animals and eggs were calculated using the three modes of the frequency distribution graphs.

Hatching time and hatching rate: Each batch of 100 eggs from small and medium size rotifers, formed during the culture was incubated under continuous lighting (white fluorescent lamp, 2000 Lux) at room temperature (26.0 ± 1.5°C) and hatching time was recorded every 30 min. The mean was taken as the hatching time.

After a culture period of 5 days, aeration was stopped and the culture was left for 10 days to promote decomposition of dead rotifers and rotifer faeces. The spent culture was decanted and the remaining culture containing resting eggs was stored at room temperature (26.0 ± 1.5°C) for a period of 15 months. Incubation of resting eggs at 5 and 15 months of storage was also carried out as above under similar conditions. Hatching time was recorded every 4 h and percentage hatching was calculated. The incubation was carried out on microwell plates (0.5 ml each well) and the newly hatched animals were cultured separately in the same wells to identify the offsprings and their eggs.

Effects of pH on rotifer population growth characteristics: Triplicate cultures at five pH levels; 5.0, 6.0, 7.0, 8.0, 9.0 and a control were used for the population growth experiments. Five 400 ml freshwater *Chlorella* cultures (4.8×10^6 cells ml^{-1}) were adjusted to the above pH levels with 0.15 M hydrochloric acid and sodium hydroxide solutions. The pH of the sixth culture which was used as control was left unadjusted. Except the control, all the other cultures were buffered with 0.15 M phosphate buffer at their respective pH values. Culture commenced with an initial rotifer density of 0.25 ind.ml^{-1} and at 25.0 ± 0.5°C under constant illumination (white fluorescent lamp, 2000 Lux) for 5 days with aeration at 0.6 l $min.^{-1}$. The following parameters were determined every 24 h taking samples from each flask to 10 well Boerner slides; total number of females, number of females carrying small eggs, number of females carrying medium size eggs and number of females carrying resting eggs. Population growth rate (G), rate of mictic female appearance (percent of mictic females among total females) were estimated according to Hagiwara *et al.*[2] Analysis of variance (ANOVA) was used to evaluate the effect of pH on population density, growth rate and the appearance of mictic females.

RESULTS

Three distinctive length and width groups within the *Brachionus calyciflorus* population and 3 groups of egg sizes were observed during the study. The mean length and width of rotifers and the mean length of eggs are given in Table 1 and the frequency distribution of these parameters are illustrated in Figure 1 a, b &c respectively. Measurements of rotifers bearing eggs indicated that rotifers with a mean width of 164.26 ± 6.54 µm bore eggs with a mean length of 80.45 ± 8.78 µm and hatched with a mean hatching time of 3.13 ± 0.78 h to give rise only to males measuring a mean total length of 88.68 ± 9.11 µm. Rotifers with a mean width of 183.06 ± 3.61 µm bore eggs with a mean length of 122.63 ± 4.87 µm, hatched with a mean hatching time of 4.01 ± 0.92 h and produced 3 groups of rotifers when propagated. Animals with a mean width of 203.62 ± 5.21 µm produced eggs with a mean length of 163.79 ± 4.64 µm (resting eggs). These eggs also hatched (after a resting period) and produced a rotifer population of all the 3 sizes.

The hatching time of resting eggs increased with age while percentage hatching decreased during storage (Table 2). The effect of pH on rotifer density, growth and appearance of mictic females are illustrated in Figures 2 a, b and 3 respectively. The maximum rotifer density was observed at pH 7 (163 ind.ml^{-1}) followed by pH 6 (115 ind.ml^{-1}) and the lowest (51 ind.ml^{-1}) in the control (pH 5.2 - 8.6). The density and growth rate differences were not significant but the appearance of mictic females at different pH levels was significantly different ($p < 0.05$). There was no growth at pH 9.0.

Table 1: Morphometric characteristics of *Brachionus calyciflorus* and their eggs formed during culture.[a]

Size Group	Rotifer		Eggs
	Mean Length (μm)	Mean Width (μm)	Length (μm)
Large	304.09 ± 1.68	203.62 ± 5.21	163.79 ± 4.64
Medium	276.44 ±14.99	183.06 ± 3.61	122.63 ± 4.87
Small	239.56 ± 8.85	164.26 ± 6.54	80.45 ± 8.78

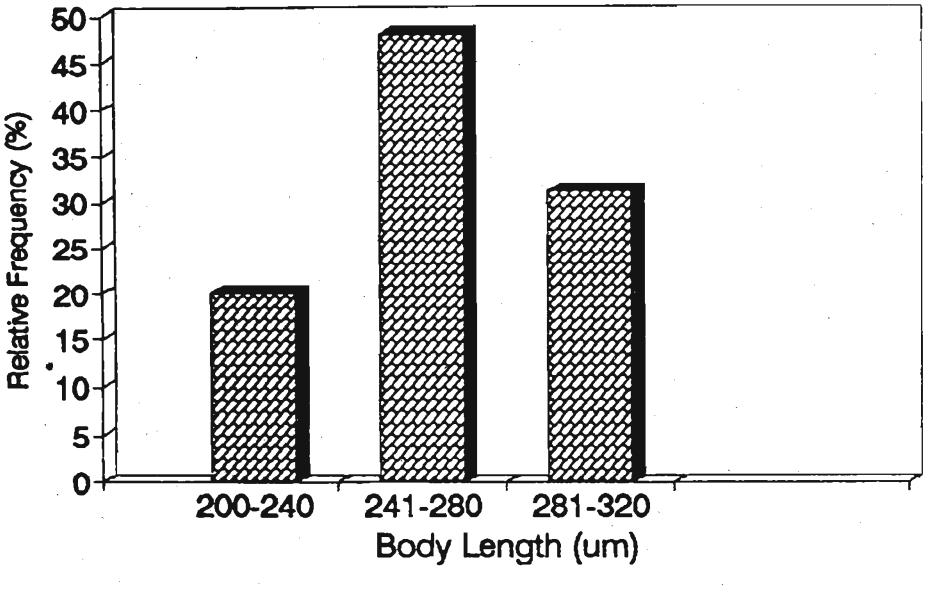
[a] At 25.0 ± 0.5°C fed on freshwater *Chlorella* sp. Mean ± SD of each mode of the frequency distribution was taken as the length and width of rotifers and length of eggs.

Table 2: Effect of storage time[a] on hatching time and percentage hatching of resting eggs of *Brachionus calyciflorus*.

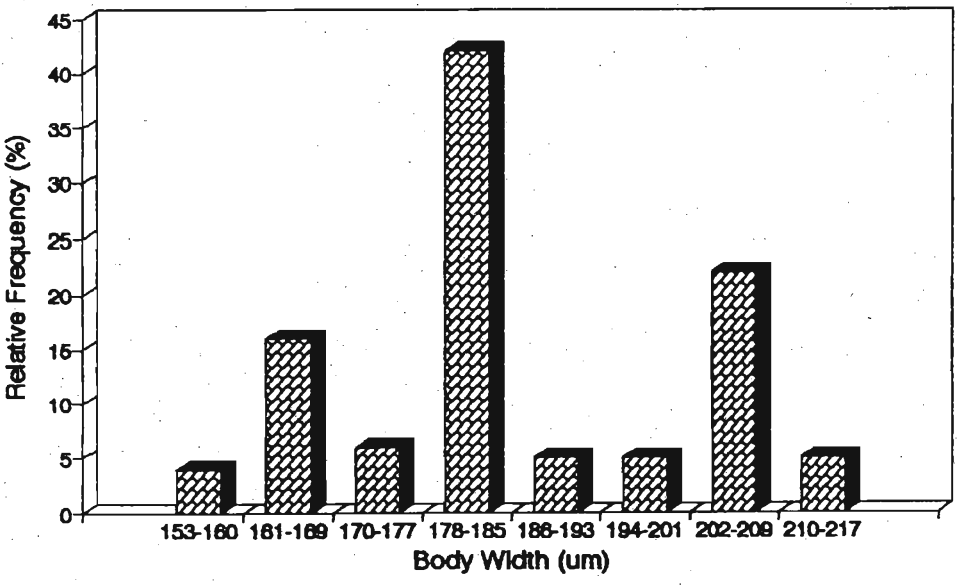
Storage Time (Months)	Hatching time[b] (h)	Hatching (%)
5.0	23.68 ± 5.96	66.60
15.0	26.61 ± 8.40	40.10

[a] At room temperature (26.0 ± 1.5°C, [b] Values are mean ± SD for 100 eggs.

(a)



(b)



(c)

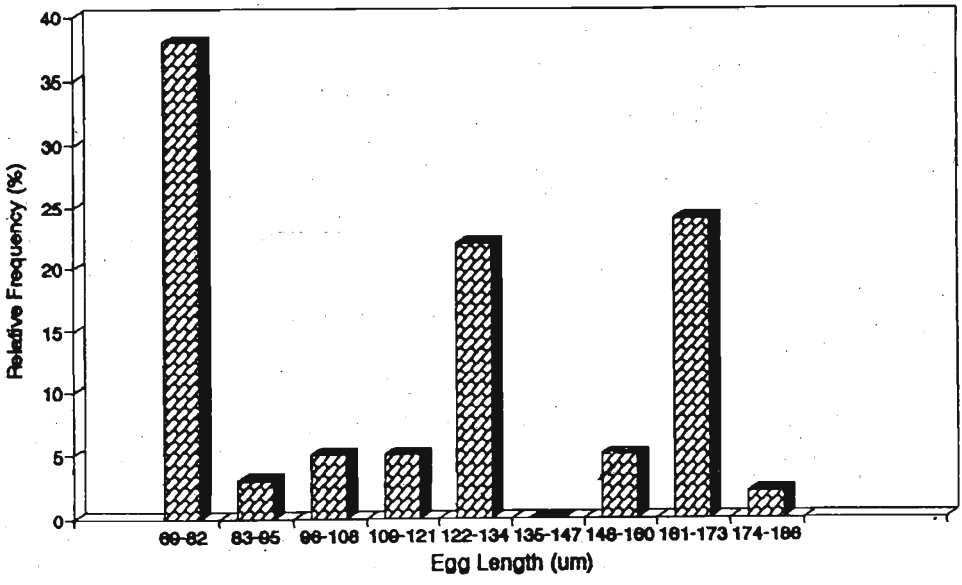


Figure 1: Frequency destribution of *Brachionus calyciflorus;* A. Total length B. Body width C. Egg length

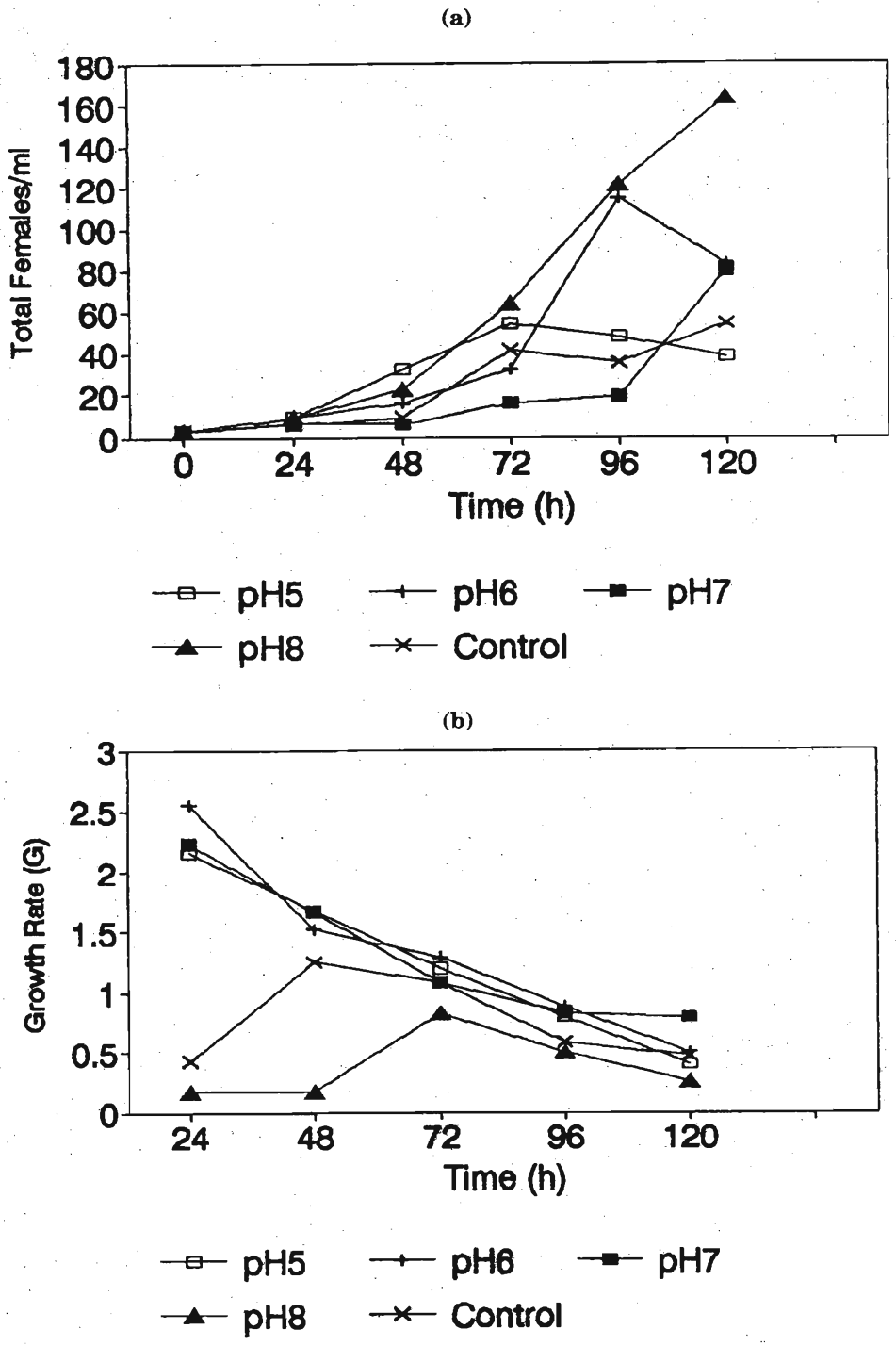


Figure 2: Population characteristic curves for *Brachionus calyciflorus*; effect of pH.
A. - Density B. - Growth rate (G)

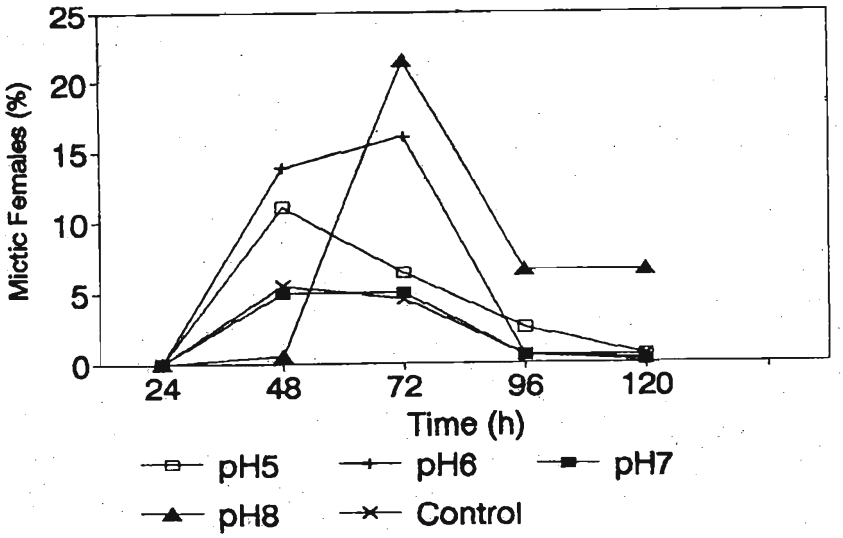


Figure 3: ***Brachionus calyciflorus*. Rate of mictic female appearance (percent of ♀♀ and D ♀ in total females) at different pH levels.**

DISCUSSION

The population of *B. calyciflorus* in the present study consisted of 3 size groups, each producing one type of eggs. Kannan and Govindasamy[7] found that the *B. calyciflorus* from Portonova, India measured a maximum length of 210 - 280 µm and width of 135 - 145 µm. These authors did not indicate the presence of different size groups in the population. Chengalath *et al.*[8] recorded an exceptionally large form of *B. calyciflorus*, measuring a total length of 490 µm and width of 420 µm in some specimens they collected from freshwater reservoirs of Sri Lanka. The size range of the rotifers in the present study falls between the ranges given by these workers. Fukusho[3] in his review stated that the size of *B. plicatilis* depends on the season; the size of this species during the cold season is about 1.3 times that of the size of rotifers during summer. Hagiwara *et al.*[2] cited the existence of genetically different, large and small size *B. plicatilis*. In the present study, the rotifers of a mean length of 239.56 ± 8.85 µm were mectic, male producing females (♂♀) and the rotifers of mean length of 304.80 ± 1.68 µm were mictic, resting egg producing females (D ♀) and rotifers with mean length of 276.44 ± 14.99 µm were amictic females (♀♀). These female symbols have been defined previously[2,9] for *B. plicatilis*. In contrast to the observations made by Fukusho[3] on *B. plicatilis*, there was an apparent difference in the appearance of ♂♀ and ♀♀ females of *B. calyciflorus* in the present study.

Tsukajima *et al.*,[2] observed a reduction in hatching time of amictic eggs of *B. plicatilis* with rise in temperature. In the present study, hatching time of amictic eggs of *B. calyciflorus* was less than that observed at 30°C for *B. plicatilis* by Tsukajima *et al.*[10] It is known that the hatching time of resting eggs of *B.*

plicatilis is 24 - 72 h, depending on age of cysts, temperature and water condition. The hatching time of resting eggs in the present study increased slightly with the storage time while percentage hatching decreased. However, Fukusho[3] stated that resting eggs of *B. plicatilis* could be preserved for more than 8 years without loss of hatchability.

The mictic female appearance is an important indicator of reproductivity of *B. plicatilis*[2] and this in turn accelerates the formation of resting eggs with subsequent reduction in the rotifer population growth because dormant embryos are produced which are temporarily dropped out of the population.[11] Extreme pH values in the present study had a significant effect on the mictic female appearance though there was no significant growth and density differences at the pH levels tested. The lowest mictic female appearance was at pH 7.0 indicating the parthenogenesis is at its highest at this pH (Fig. 3). This could probably be explained by the fact that the modified pH would trigger the population to shift to sexual reproduction. Snell[12] compared the population growth rate of *B. plicatilis* with sexual reproduction rate and concluded that parthenogenesis and sexual reproduction are affected differently by extreme environmental conditions. Hagiwara *et al.*[2] stated that an increase in mictic females could suppress the population growth rate of *B. plicatilis*. The optimum pH range observed in the present study (5.0 - 8.0) is partly in agreement with the observations of Hoff and Snell.[1] They reported an optimum pH range for *B. calyciflorus* at 25°C as 6.0 - 8.0 with upper and lower limits of 9.5 and 4.5 respectively. With the lowest mictic female rate at pH 7.0, the maximum population density achieved in the present study was far less than the densities obtained by Schluter and Groenweg[13] when they cultured *B. ruben*, another freshwater rotifer species, in algal production ponds to treat piggery waste.

From the present study it is concluded that the population of *B. calyciflorus* consists of 3 size groups, each producing a particular type of eggs. The optimum pH range for growth was 5.0 - 8.0. When the pH deviates from the neutral, the population shifts to sexual reproduction as a response to the change in the environment. This shift could easily be detected by the increased ratio of mictic females to amictic females.

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