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Effect of Microplastic on lifetable attributes of *Ceriodaphnia cornuta*: a laboratory bioassay test

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Abstract

Ubiquitous presence of microplastics (MPs) in present age causing toxicity throughout the trophic level in aquatic ecosystem. Here, we studied life table attributes of *Ceriodaphnia cornuta* in MPs contaminated environment. Life table parameters were estimated at 1 mg L^{-1} concentration of MPs against control. We studied longevity, fecundity, maximum life span, net reproductive rate (NRR), gross reproductive rate (GRR) and intrinsic rate of natural increase among treatment and control cohort. The present results indicate that MPs negatively affected the survival and fecundity of *C. cornuta* at environmentally relevant concentration. Overall our studies show that acute exposure to MPs has no impact whereas chronic exposure has high impact on *C. cornuta.*

Key words : Microplastics, *Ceriodaphnia cornuta*, life table parameter

Plastic is widely used in the Anthropocene due of its light weight, strength, and comparatively lower production cost[33]. Each year, over 8 million tonnes of plastic trash are carried from land to sea,[15] making up globally 73% of all marine trash[1]. Microplastics (<5mm)[10] are considered to be the most prevalent type of plastic contamination in the marine environment[4]. Due to its size, abundance[7], and higher bioavailability than macroplastics[31] microplastic has been identified as a concern to the marine ecosystem. Microplastics of the size range 63-32 um are readily ingested by the freshwater organism (*Physa acuta*) and affects its reproduction and locomotion rate[18]. Plastics can be consumed directly or by the consumption of contaminated prey, direct consumption is either intentional, when plastic items are mistaken for prey or unintentional when plastics are ingested passively[27]. Zooplankton can easily consume microplastics[2]. There have been reports of zooplankton in the Northeast Pacific Ocean consuming microplastics, highlighting the need for investigations on their toxicity[6,11]. Planktonic animals' behaviour can change after ingesting

MPs; examples include *Daphnia magna* becoming immobile[29] and *Cyprinodon variegatus* larvae swimming more slowly[4] this can shed light on the possible negative impacts of microplastics on organisms at lower trophic levels. Furthermore, the discovery of microplastics, especially fibres and beads, in freshwater environments like rivers, lakes, and estuaries highlights the necessity for toxicity research using freshwater species[3,8,35]. *Ceriodaphnia cornuta* is one of the most prevalent representative of cladoceran species[5], and it can be found in many shallow lakes and eutrophic ponds in tropical and subtropical regions. Cladocerans are one of the most significant biological group of the zooplankton communities that contribute to the energy and matter transfer in freshwater ecosystems[32].

Numerous experiment has been conducted on zooplankton assessing the impact of MP on reproduction, mortality and growth but there is lack of information on complete life table parameter. In this study I examined the toxicity of common available MP in aquatic ecosystem namely polystyrene of size 20-32 µm following acute and chronic bioassay against freshwater zooplankton (*C. cornuta*) with a focus on life history trait such as longevity, Fecundity, age at first reproduction, generation time, life expectancy, maximum life span and Intrinsic Rate of Natural increase (r_{max}).

Microplastic preparation and size determination:

Large pieces of plastics were bought from plastic factory. These large plastics were grinded in mixer (Bajaj Rex 750 W) to make it fine particles. Grinded plastics were passed through the sieve to get the desired size of microplastic. Microplastic of size range 32-20µm were used in the experiment.

Test organisms: The zooplankton samples were collected from pond of Central University of South Bihar (25°35' 51 "N 85°05' 15"E), Gaya, Bihar, India. From collected sample healthy *C. cornuta* was isolated and mass culture was established in 1000 mL beakers using autoclaved tap water as culture medium and *Chlorella vulgaris* (2.5×10^6/ mL) as food. The culture medium was changed at the interval of 24 h and maintained at 25 ±1.5°C temperature. Neonates used in the experiment were less than 24 h old.

Experiment I (acute bioassay): This acute toxicity test was conducted using four different concentrations (0,0.25,0.5 and 1.0 mg L^{-1}) of MPs. This bioassay test was conducted to select the right concentration of MPs for the chronic bioassay test. The experiment was conducted in 50 mL glass beaker containing 20 ml of test solution for all the four concentrations in quadruplicate. A cohort of five individuals of *C. cornuta* was introduced in pre-assigned beakers. Experimental set up were incubated for 48 ±1.5 h at 25 ± 2 °C. After 48 ±1.5 h of incubation, at each concentration live and dead individuals were counted carefully under the stereo zoom microscope (MAGNUS).

Chronic toxicity tests : By using values from acute bioassay test appropriate concentration (1mg L^{-1}) of MPs has been selected for chronic toxicity test. Experiment was conducted to understand the impact of selected concentration of MP on life table parameter (Experiment II) of *C. cornuta*.

Experiment II (Life table parameter): The present work estimates the life table parameters in *C. cornuta* exposed to 1 mg L^{-1} concentration of MP against the control. The experiment was conducted in 50 ml beaker

containing medium (autoclaved tap water with *C. vulgaris* as food) for control and treatment (MP + *C. vulgaris* as food). Each of the beakers was introduced with 5 neonates (<24 h). All the experiment was conducted in quadruplicate. Each day at the interval of 24±1 h live *C. cornuta* from original cohort was counted neonates and dead adults Ire discarded if found any. Surviving adults Ire transferred to new pre-assigned beakers containing medium and food. This experiment was terminated when every individual of each cohort was died. By using standard life table method Age-specific survivorship (l_x) and fecundity (m_x) for each cohort were calculated[25]. In addition, I calculated other life table parameter like; life expectancy (equation 1), the average lifespan (at lx = 0.5), the gross reproductive rate (GRR, equation 2), the net reproductive rate (NRR, equation 3), generation time (GT, equation 4) and the intrinsic rate of population increase (equation 5).

Life expectancy:

$$e_x=\frac{T_x}{n_x} \quad (1)$$

Gross Reproductive Rate:

$$GRR=\sum_0^\infty m_x \quad (2)$$

Net Reproductive Rate, R_0:

$$R_0 = \sum_{r=0}^{\infty} l_x m_x \quad (3)$$

Generation Time:

$$GT=\frac{\sum_0^\infty l_x m_x x}{R_0} \quad (4)$$

Intrinsic Rate of Natural increase (r_{max}): after solving Euler-Lotka equation

$$\sum_x^\infty e^{-rx} l_x m_x=1 \quad (5)$$

Statistical analysis : Obtained data of Gross Reproductive Rate, Net Reproductive Rate, 50% longevity, life expectancy at birth (Ex), generation time (GT) and age at first reproduction were subjected to One Way ANOVA followed by post hoc Student-Newman-Keuls test (SNK). To reduce heteroscedasticity Euler's r data were arch sign transformed prior to use. All the analyses were carried out on SPSS software (version 21.0).

Experiment I (dose response curve): No mortality occurred in 0.25, 0.5 and 1.0 mg L^{-1} MPs concentrations provided in 48 h of exposure treatment.

Experiment II (Life table estimation): Microplastics affected all the life table parameter of *C. cornuta*. Median longevity recorded 25.53% shorter in the microplastic treated environment (Fig. 1). In control both life expectancy at birth and Median longevity were significantly higher ($p< 0.05$ One-way ANOVA) than treatment (Fig. 2, Fig. 3). *C. cornuta* did not record any difference in maturity but showed longer generation time in treatment than in control (Fig. 3). In treatment net, gross reproductive rates and intrinsic rates of natural increase were significantly lower ($p<0.05$; ANOVA; Fig. 4) than in control. Microplastics applied environment incurred 63.97% and 60% decrease in net and gross reproductive rate respectively and 29.76% reduction in intrinsic rate of natural increase (Fig. 4).

Since *C. cornuta* has relatively shorter life period a life table study was ideal to assess the sublethel impact on demographic parameters. In present study no mortality was observed during acute toxicity (48 h) test at any of the concentrations (0.25, 0.5 and 1 mg L^{-1}) in *C. cornata* like other study in *D. magna* at concentration 25- 250 mg L^{-1} for the same duration[12,21,26,29]. Puranen Vasilakis[26] revealed that *D. magna* on exposure to microplastics of size 1-5 µm and at concentration of 2 mg L^{-1},

30% mortality happens whereas in another study conducted by Pacheco *et al*.,[24] on same species same microplastic size but at lesser concentration (0.65 mg L^{-1}) observed 26% mortality. However, in present study I recorded 100% mortality in microplastics exposed concentration (1mg L^{-1}) and 30% in control on 19th days. Reduced survival may be due to the aggregates formation of microplastic which might do internal damage while passing through gut or create hindrance during swimming[34]. Polystyrene microplastic of size 0.1 µm did not pose any effect on survival of rotifers (*Brachionus plicatilis*) after 24 and 48 h of exposure at concentrations (0.01–10 mg L^{-1})[9] in contrast *Brachionus koreanus* showed decreased survival of ~1.6 days than control after exposing at higher concentrations (1, 10 and 20 mg L^{-1}) and larger size of 0.5 µm[16]. Among all the zooplankton studied (gastropod, euphausid, barancles,bivalves, ascidians, brine shrimp and rotifers) Sea urchin and daphnids are most affected than other species at concentration > 1mg L^{-1} but not at concentration generally found in the environment (0-1 mg L^{-1})[34].

Microplastic shows negative impact on reproduction rate of different zooplankton. *Tigriopus japonicus* when exposed to microplastic (size=0.5 and 6 µm) at concentration 0.1–25 mg L^{-1} should decrease in offspring production of about 56-72% in respect to control[19] whereas *Paracyclopina nana* at concentration 0.1–20 mg L^{-1} showed decrease of about 12-24% [17]. *D. magna*, *D. pulex* and *C. dubia* when exposed to microplastic shoId significant decrease in offspring number of about 9%–94%, 26%–46% and 24%–65% respectively[14,22,24,26,36]. In present study we

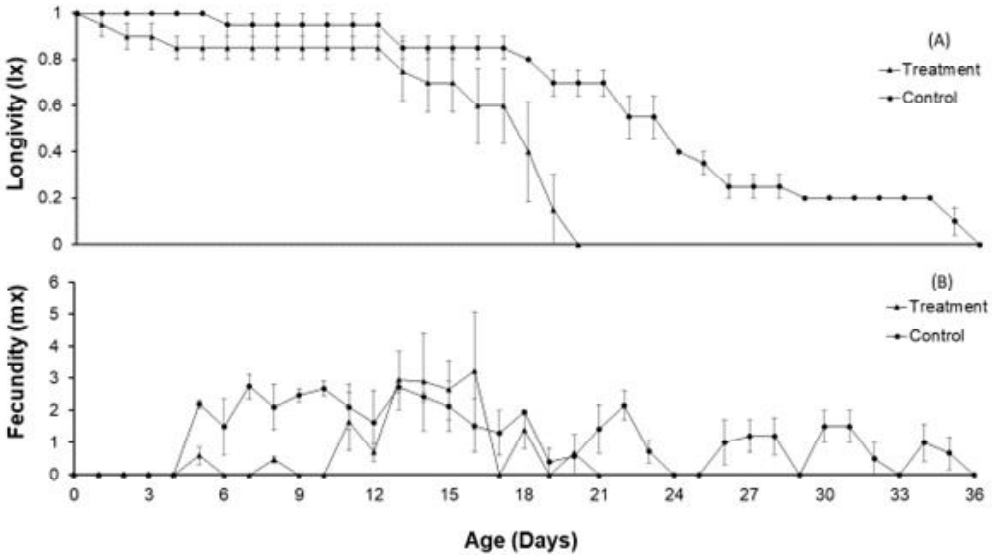

Figure 1. Age (x) specific survivorship (A) and fecundity of *C. cornuta* (B) in treatment and control. Every data point shows mean and standard error values of all four replicates with initial cohort size five in each.

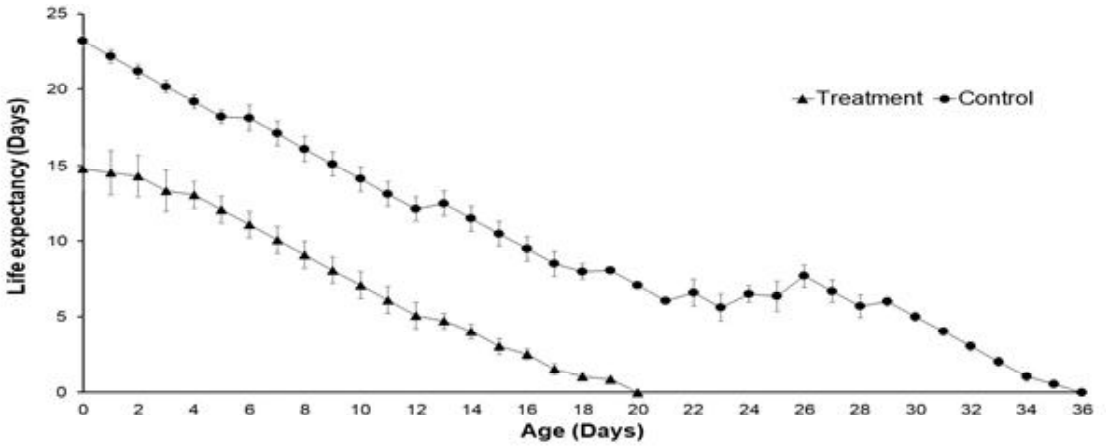

Figure 2. Age (x) specific life expectancy (Ex) of *C. cornuta*. Every data point shows mean and standard error values of all four replicates with initial cohort size five in each.

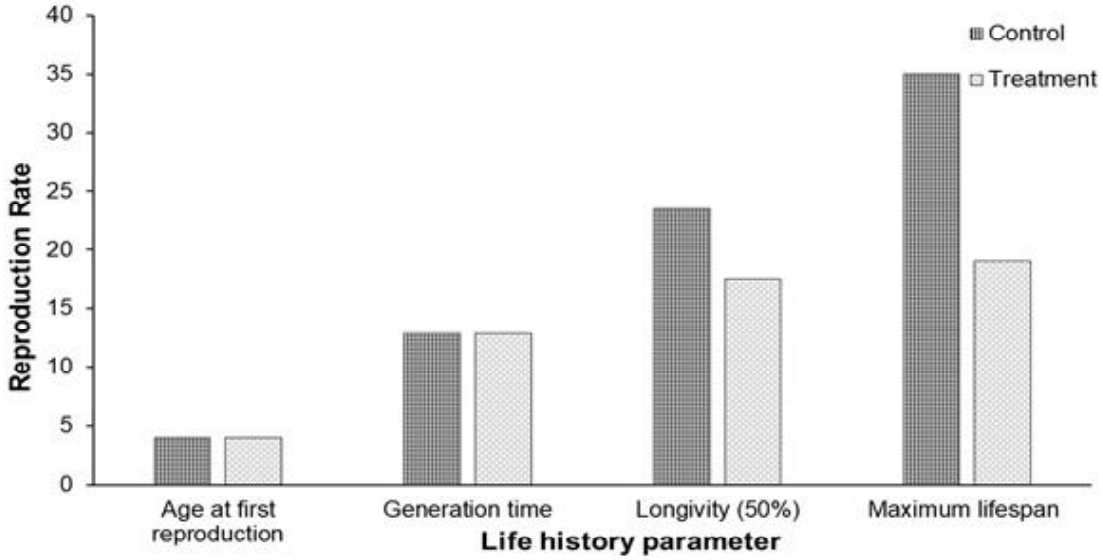

Figure 3. Survival related life table demographic parameter of *C. cornuta* in treatment and control.

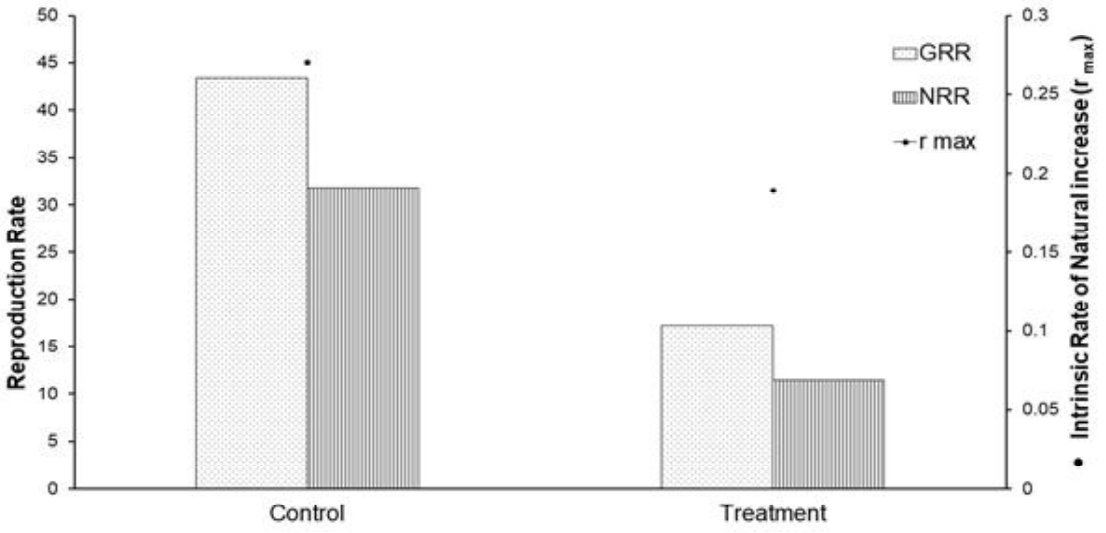

Figure 4. Reproduction related life table parameters (Gross Reproductive Rate, Net Reproductive Rate and Intrinsic Rate of Natural increase (r_{max}) of *C. cornuta* in treatment and control.

observed decrease in fecundity (GRR) of about 60% in microplastics exposed environment. Ziajahromi *et al*.,[36] in his study observed 20%–80% reduced fecundity in *C. dubia* hoIver in *D. magna* no reduced fecundity observed[13]. Liang *et al*.,[20] conducted study on rotifers found decrease in reproductive ability with increasing concentration and time of microplastic exposure. At concentrations 0-1 mg L^{-1} (environment relevant concentrations), fecundity decreased about 6-12% whereas at concentration >1 mg L^{-1} (higher than environment) fecundity decreased 30-57% of zooplankton (copepods, daphnids, brine shrimp and rotifers) after Microplastic exposure[34]. This may be due to less ingestion of natural food in plastic contaminated environment resulting in less availability of energy for the reproduction. In most studies conducted on *D. magna* impact of microplastic was not visible on maturity (age at first reproduction)[14,22,23,30]. Similar to finding of other studies in the present study also there was no impact of microplastic was recorded on maturity (age at first reproduction).[23] in his study observed impact on generation time of *D. magna.* In present study I observed increased generation time in *C. cornuta.* Severity of microplastics toxicity depends upon its type, size and concentrations and species specific zooplankton. Microplastics poses impact on reproduction and survival of zooplankton.

MPs causes higher mortality and lesser fecundity in *C. cornuta.* However, it does not cause mortality within 48 h but its impact can be seen with increasing time in term of longevity, fecundity, generation time and

intrinsic rate of natural increase. MPs exposure at environmentally relevant concentration decrease overall fitness of *C. cornuta.*

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