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Survivorship Curves for Cyclops (*Mesocyclops leuckarti*, *Mesocyclops hyalinus*) in Response to Varying Urea Concentration.

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Abstract:
Agriculture and industrialization are central to economic growth but have also intensified environmental challenges, particularly through the release of agricultural runoff and industrial effluents into freshwater bodies. Urea and ammonia-based fertilizers are among the most prevalent contaminants in these ecosystems. This study evaluates the acute toxicity of urea on freshwater zooplankton belonging to the genus *Mesocyclops*. Survival responses were assessed by exposing groups of 50 individuals to 100 mL urea solutions at concentrations of 0.12 M, 0.08 M, 0.04 M, and 0.01 M for a duration of 264 hours (11 days). A clear concentration-dependent decline in survivorship was recorded. The control group exhibited a Type I survivorship curve, whereas exposed groups (0.01 M–0.12 M) showed a shift toward Type II patterns. Females bearing egg sacs failed to hatch at higher urea concentrations, and reduced motility was evident across all treatment sets. These results underscore the negative ecological implications of urea pollution and its potential to disrupt freshwater zooplankton populations.

Key Words: Urea, *Mesocyclops leuckarti*, *Mesocyclops hyalinus*, Zooplankton, Survivorship curve.

Introduction:

Agricultural and industrial sectors are expanding rapidly due to the growing population, which raises the need for water necessary to support all life on this blue planet. Agricultural areas, industry, and human and animal consumption rely on rivers, groundwater, and lakes as their primary water supplies. The quantity and quality of drinkable water have declined significantly due to rising water contamination from waste discharged from industry, agriculture, homes, towns, and other sectors. (Ahmed et al., 2021) Industrial pollutants, sewage water, and contaminated wastewater from contaminated sources can negatively impact ground and surface water quality. Without proper treatment, a significant portion of industrial effluent is released, causing harm to human health and potentially contaminating underdeveloped nations' aquifers. Wastewater from urea fertilizer factories releases harmful heavy metals and extreme physicochemical parameters. (Laghari et al., 2018)

The use of fertilizer has boosted global plant yields and helped fight hunger. However, the overuse of fertilizers has detrimental impacts on the environment and public health if it does not lead to increased agricultural output. Significant levels of nitrogen accumulate in the soil due to nitrogen over-fertilization. Leaching and denitrification result in a substantial amount of nitrate being lost, in addition to uptake by plants. This situation is harmful to both the economy and ecology. (Hashimi & Hashimi, 2020)

Long-term, careless fertilizer usage has grown to be a major cause of soil and water contamination, endangering human health and downstream pristine terrestrial and aquatic ecosystems. Heavy metals (HMs), including cadmium (Cd), mercury (Hg), arsenic (As), chromium (Cr), lead (Pb), and others, are naturally present in soils; however, overuse of fertilizers exacerbates the condition by reducing the pH of the soil. (Khan et al., 2017) Around the world, urea is one of the most widely used nitrogen fertilizers. It has a very high bioavailability and a high nitrogen concentration (46%). The primary cause of atmospheric ammonia emissions is agriculture. 80–95% of the gas's overall emissions are caused by it, with industry and the burning of fossil fuels accounting for 0.7%, animal husbandry for 48.6%, biomass combustion for 13.3%, and mineral fertilizers for 20.3%. (Skorupka & Nosalewicz, 2021) Edible oils like mustard, groundnut, sesame, and soybean were used to create copper (II) soaps. These soaps were then complexed with urea in a 1:1 molar ratio, resulting in urea soap or copper soap-urea complexes. These complexes, containing urea ligand, have stronger antifungal properties than copper soaps alone, making them known as "urea soaps."(A. K. Sharma et al., 2018) Plankton, a significant part of natural waterways, play a crucial role in biogeochemical cycles and contribute to the biological transformation of other species and sediments. (Walsh & Environmental Research Laboratory, United States Environmental Protection Agency, 1977)

When environmental factors or agricultural practices inhibit the microbial breakdown of urea in soil, fertilizing agricultural fields with urea promotes urea export to aquatic habitats. Urea was used sparingly and locally until 1970, but since then, its usage has grown rapidly, and it now makes up 50% of all N fertilizer applications worldwide. (Finlay et al., 2010)One of the secondary excretory products of marine zooplankton is urea. (Carpenter & Capone, n.d.) Zooplankton are essential for the movement of particulate organic materials from the surface to deeper waters as well as the cycling of dissolved organic and inorganic substances. (Saba, 2009/2010)

A vital part of aquatic food webs, zooplankton play a crucial role in biogeochemical cycling and the movement of matter and energy from primary producers to higher trophic levels. Different environmental conditions influence different types of zooplankton. (Karpowicz & Ejsmont-Karabin, 2021) Cyclops can adapt to a variety of environmental conditions. While some are suited to damp environments, it may be found in salt marshes, ponds, lakes, estuaries, and marine sites—its natural home. Zooplankton Cyclopes were able to proliferate in water bodies, particularly freshwater lakes and reservoirs. Cyclops are thought to be the most prevalent species in freshwater and marine environments with a high organic matter concentration. (Alsalman et al., 2009)

Copepods may be found in nearly every freshwater ecosystem, including the biggest ancient lakes, underground waterways, and pools of glacial melt water, hot springs, hyper-saline lakes, and phytotelmata. In freshwater, they are incredibly prevalent and play a significant role in the majority of planktonic, benthic, and groundwater communities. The marine habitat has the most diversity of copepods, with an estimated 13,000 morph species recognized, whereas freshwater is home to about 2,814 species. (Boxshall & Defaye, 2007)
Numerous environmental conditions influence copepod size, which varies greatly. The morphometric variability of several copepod species has been investigated in various water bodies worldwide. (Can & Bozkurt, 2019) The Cyclopidae is the biggest group within the subclass Copepoda and the most species-rich family (around 830 species) among copepod lineages that reach continental waters. (Wyngaard et al., 2010) Among the Cyclopidae family, the genus *Mesocyclops* 1914 is regarded as a worldwide group; yet, it is more prevalent in eutrophic water bodies and more dominant in the tropics. (Luong et al., 2020)

The head and thorax fuse to form the cephalothorax, which has complex eyes and sensory antennae. The abdomen is made up of many sections and usually has two furcal rami, which resemble tails. *Mesocyclops* is Long and segmented; its antennae are utilized for swimming and environmental sensing. *Mesocyclops* have adapted legs for swimming and movement, typically with setae to enhance surface area. The size and reproductive systems of men and females differ. Eggs are placed in egg sacs, which may be attached to substrates (female legs) or free-floating. *Mesocyclops* is frequently found in freshwater habitats, such as lakes, reservoirs, and ponds. (Luong et al., 2020)

Materials and Methods:

i. Isolation of Cyclops:

The Copepods used for the experiment were collected from the pond water of Sathaye College, Mumbai. These zooplanktons were collected using a plankton net and were transferred to a container having distilled water. Zooplankton such as Planaria, Daphnia, Hydra, Mosquito larvae, *Aleosomes*, Copepods, etc, were present. From these, Copepods were isolated manually with the use of pipettes and droppers. The Cyclops were given Algae for consumption. They were subsequently kept in an adequate amount of light exposure.

ii. Preparation of Urea Concentrate:

A stock solution of 0.24M was prepared using 7.207 g of Urea in 500 mL of Distilled water. Using the stock solution, serial dilution was done in the following ratio:

- 0.24M was diluted in the ratio of 1:1 to make 0.12M of urea solution.
- 0.24M was diluted in the ratio of 1:2 to make 0.08M of urea solution.
- 0.24M was diluted in the ratio of 1:5 to make 0.04M of urea solution.
- 0.24M was diluted in the ratio of 1:7 to make 0.01M of urea solution.

Therefore, the concentrations of urea, which are 0.12M, 0,08M, 0.04M, and 0.01M, were used to examine the survival rate of Copepods.

iii. Experimental Setup:

The experiment was carried out at 32℃ and 62% humidity, which was assessed using a hygrometer. In each beaker, 50 *Mesocyclops sps.* (Copepods) and 100 mL of 0.12M, 0.08M, 0.04M, and 0.01M concentration of urea was added, respectively. In each beaker, algae were added for consumption. This experiment was carried out in triplicate to find the Standard deviation. The experiment was tested for 24hrs, 48hrs,72hrs, 96 hrs, 120 hrs, 144 hrs, 168 hrs, 192 hrs, 216 hrs, 240 hrs and 264 hrs, to evaluate the survival rate of Cyclops on urea.

Results:

Table 1: No. of Cyclops Alive (0hrs- 72hrs)

Concentration	0hr	24hrs	48hrs	72hrs
Control	50±0	50±0	49.67±0.33	48.33±0.33
0.01M	50±0	48.67±0.33	44±0.58	27±0.58
0.04M	50±0	46.67±0.33	33.67±0.88	23±0.58
0.08M	50±0	44±0.58	28±0.58	17.67±0.67
0.12M	50±0	41.67±0.88	24±0.58	14±0.58

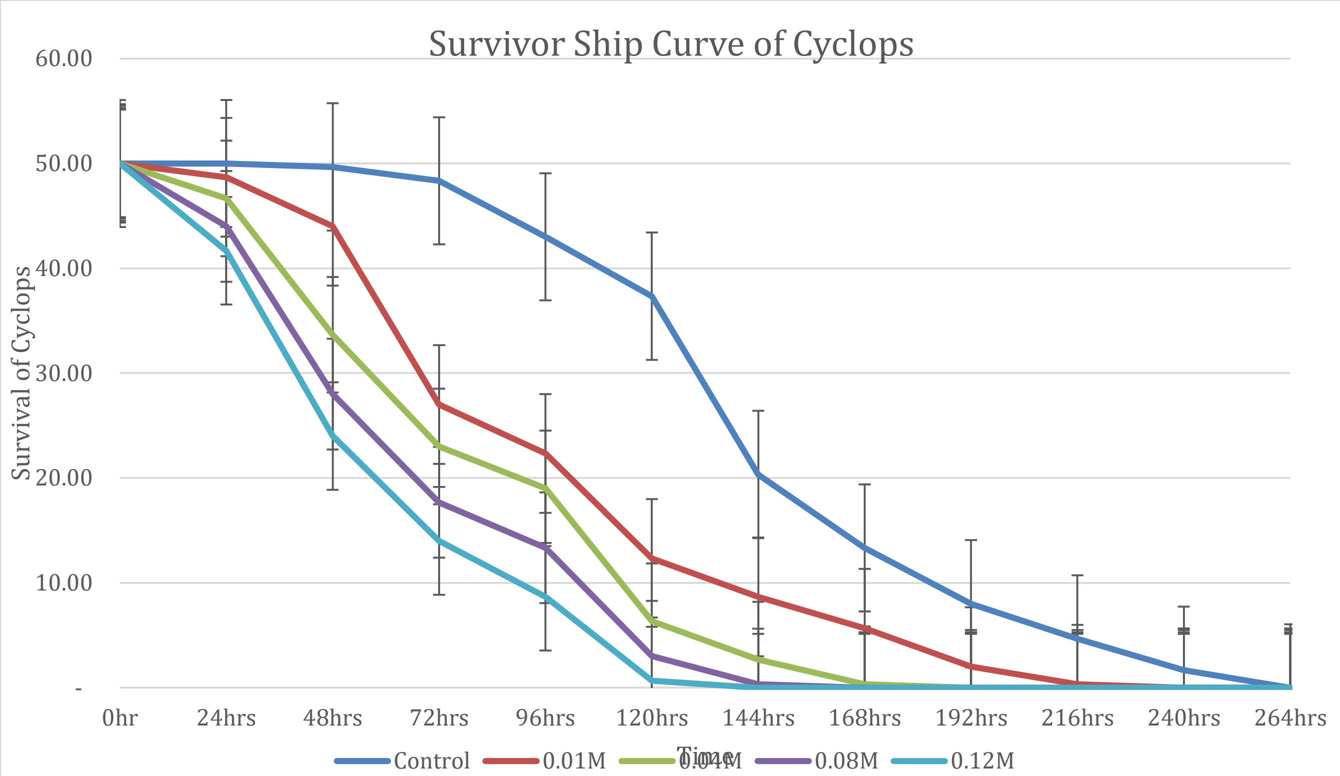
Table 2: No. of Cyclops Alive (96hrs- 168hrs)

Concentration	96hrs	120hrs	144hrs	168hrs
Control	43±0.58	37.33±0.33	20.33±0.33	13.33±0.33
0.01M	22.33±0.33	12.33±0.33	8.67±0.33	5.67±0.33
0.04M	19±0.58	6.33±0.33	2.67±0.33	0.33±0.33
0.08M	13.33±1.2	3±0.58	0.33±0.33	0±0
0.12M	8.67±0.33	0.67±0.67	0±0	0±0

Table 2: No. of Cyclops Alive (192hrs- 264hrs)

Concentration	192hrs	216hrs	240hrs	264hrs
Control	8±0.58	4.67±0.33	1.67±0.33	0±0
0.01M	2±0.58	0.33±0.33	0±0	0±0
0.04M	0±0	0±0	0±0	0±0
0.08M	0±0	0±0	0±0	0±0
0.12M	0±0	0±0	0±0	0±0

Fig.1: Survival Ship curve in varying concentration of Cyclops.



In Control setup a Concave declining graph was obtained with deviations ranging from 72 hrs. A steep decline in the population of Cyclops was observed after 120hrs of exposure, later a gradual decline in the population was observed. Here, a Type 1 Survivor ship curve was observed.

In 0.01M, 0.04M, 0.08M and 0.12M concentration, a Type 2 Survivor ship curve was obtained. Following all the concentrations rapid decline in the population of Cyclops was seen with increasing concentration and exposure period.

Discussion:

Copepods may be found in nearly every freshwater ecosystem, including the biggest ancient lakes, underground waterways, pools of glacial meltwater, hot springs, hypersaline lakes, and phytotelmata. In freshwater, they are incredibly prevalent and play a significant role in the majority of planktonic, benthic, and groundwater communities. (Boxshall & Defaye, 2007) The Cyclops are mainly found in freshwater and are considered an indicator species for freshwater ecosystems. The Cyclops were isolated from the ponds that were found in the Euphotic zone (the uppermost surface of the pond that receives maximum sunlight).

Overuse of pesticides has been an increasing concern, as it largely contributes to urea contamination in the water bodies, which leads to biomagnification. Increased offspring mortality rates and prolonged embryonic development periods were caused by pesticide exposure. When exposed to pollutants, the quantity of eggs deposited also decreased drastically; the combined impact of both pesticides was more severe than either one alone. ("Survival and Reproduction of Cyclops Abyssorum (Freshwater Copepod) Exposed to Spirotetramat and 2,4-D," 2018) Egg containing Cyclops were added for the experimental setup. These egg-containing Cyclops did not survive in the urea solution after 48 hours of exposure. The Cyclops were unable to hatch their eggs due to the urea present in the solution; hence, the population size did not increase. The egg containing cyclops did not sustain the higher concentration of urea, as a steady population was observed primarily, but in the increased exposure period, the failure in hatching of eggs was seen. As the amount of urea increased in the water solution, it relatively succeeded the density of water, along with the addition of urea, causing significant pH and Biochemical variation in the water.

Environmental factors can affect NH_3-N'ssurvival rate, affecting their developmental characteristics and reproductive capacities. (Han et al., 2022). As ammonia restricted the growth and development of Cyclops, *Mesocyclops sps.* Could not survive when exposed to varying concentrations of urea (a derivative of ammonia). Prominent observations include, in 0.01M concentration Type 2 survival ship curve was obtained, where the higher possibility of survival with a steady population size was seen, but the curve indicates the slow and gradual decline in the population of cyclops. The gradual decline of population was mainly influenced due to lack of reproduction in the Cyclops species, as the Cyclops had a reduced capacity for hatching eggs due to an increase in the exposure period of urea.

Whereas, in 0.04M concentration, a Type 2 survival ship curve was obtained, where the restrained decline in the population was perceived. The population size remained changing for a prolonged period of time, and a steady decline in the population was observed.

In 0.08M concentration and 0.12M concentration, a Type 2 survival ship curve was observed, where an expeditious decline of population was observed as the highest concentration of urea was used, which caused a low survival rate as extensive urea contamination and degradation of water quality occurred. Due to this, the descending graph was obtained with the possible lowest survival rate of Cyclops.
The findings of this research indicate that the lowest survival rate was prominently observed in 0.12M of Urea concentration.

The Cyclops exhibited a decrease in motility rate; also, the female egg-carrying Cyclops were unable to hatch their eggs due to the presence of a higher amount of urea in all the solutions. Therefore, it can be stated that the increase in industrialization and modern agricultural practices contributes significantly to the release of urea into freshwater bodies through industrial discharge, the release of fertilizers, and faecal matter. In recent studies, it was also found that many soaps contain some percentage of urea; human bathing activities in freshwater bodies lead to the release of urea in water due to the release of bodily excretions. (Sharma et al., 2018). All of these practices have resulted in causing potential decline and harm to the population of Cyclops in freshwater bodies. However, further studies can be carried out to evaluate the different parameters on various other Zooplanktons.

Conclusion:

In the experiment carried out, in the Control setup Type 1 survival ship curve was observed and in 0.01M, 0.04M, 0.08M and 0.12M concentration a Type 2 survival ship curve was seen.

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Conflict of Interest:

There is no conflict of interest.

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