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Plankton diversity of a freshwater perennial pond of northeast India with an additional report of freshwater tardigrade for India

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Abstract
A freshwater perennial pond Jorhat district of Assam was investigated for nine months during the year 2019 to gather preliminary information on the assemblage pattern of phyto and zooplankton. A total of 99 plankton species was recorded during the study of which 58 were phytoplankton and 41 belonged to zooplankton. Phytoplankton community was composed with 41.38% Chlorophyceae, 37.93% Bacillariophyceae, 10.34% Euglenophyceae, 8.62% Cyanophyceae and 1.72% or single species of Dinophyceae. Diatom genus *Navicula* was the speciose one. Zooplankton community dominance was Protozoa (31.71%) > Rotifera (29.27%) > Cladocera (17.07%)> Copepoda (14.63%). Three arthropod zooplankton, not belonging to these categories were grouped as Insecta which contributed 7.23%. One of the significant findings during the investigation was the presence of Freshwater Tardigrade in the pond, in the situation where global information on freshwater Tardigrade is scanty and in Indian context, it is close to zero. This report provides the first photographic documentation of Tardigrade from Northeast India.

Keywords: plankton, freshwater pond, Tardigrada, Jorhat, Assam, northeast India

Introduction
Planktons are some minute uni- or multicellular drifting aquatic organisms of both plant and animal origin, which have limited mortality, but influenced by water currents. Plankton have been attracting researchers of various fields as they contribute primary and secondary productivity of an aquatic ecosystem [40], serve as food for fish and aquatic organisms [31], as bio-indicator [17, 44], in nutrient cycling [43], maintaining food chain [25], regulator of water quality [21]. The abundance, density and dynamics of plankton in water body may be influenced by various factors [32, 2, 50]. Various studies on freshwater plankton in different parts of India have been carried out in the recent years [3, 46, 47] etc. In the Northeast Indian state Assam also, studies on phyto and zooplankton have been carried out in different rivers, wetlands and ponds [1, 9, 10, 29, 33, 41]. Except few, such studies are confined to Barak Valley of southern Assam and Deepor wetland of Kamrup which is not sufficient for a state having a very large amount of natural water bodies. In Jorhat District, the only available earlier study was Bordoloi [5] who investigated the phytoplankton community structure in a wetland ecosystem. No studies on plankton in man-made aquatic ecosystems like pond have been carried out in Jorhat and neighboring districts. Therefore, a preliminary study was conducted in a perennial pond to understand the pattern of diversity and composition of plankton species.

Material and Methods
Study site: The study was carried out the perennial pond present on the campus of Bahona College (approximately 26.8100° N, 94.2408° E) located 15 km North to Jorhat town of Assam, Northeast India. The size of the pond was 40m x 12m with an average depth of 2.5 m during the post monsoon season. It is a rainwater fed pond with earthen bank. During the study period, it was partially covered with floating, submerged and rooted vegetation.
Collection of Plankton: Plankton samples were collected twice a month randomly from different parts of the pond using plankton net during the period from March to November of 2019. Samples from un-accessible mid part of the pond were collected by sieving the water by attaching the plankton net to a long bamboo rod. A medium sized glass test tube (20 ml capacity) was attached at the bottom of the net where the plankton samples were collected.
Fixation and Preparation: About half part of each collected samples were preserved using 4% formalin solution while the rest was used to observe plankton in live condition. The samples for examination were gently mixed and centrifuged at a lower RPM at for 10 to 15 seconds, so that plankton precipitate as well as maintain their structural integrity. Temporary mounts of the precipitated sample were prepared and observed under different magnifications of bright field microscope (Olympus Magnus HM-100). Staining agent eosin, methylene blue, safranin were also used in some instances.
Identification: Identification of plankton were done using standard literature of Needham and Needham [35], Edmondson [20], and Patterson [39]. Identity of individual type of plankter is limited upto Genus and multiple species under a genus is marked with sp1, sp2 and so on. Photographic record and verification were also made using android phone camera (16 Megapixel sensor) using a phone to microscope adapter.

Results
A qualitative study of both the phyto and zooplankton were done during the period. Among the phytoplankton, Chlorophyceae was found to be dominant with 41.38%, followed by Bacillariophyceae (37.93%), Euglenophyceae (10.34%), Cyanophyceae (8.62%) and Dinophyceae

(1.72%) which is shown in Fig (Fig. 1). Altogether 58 species of phytoplankton were recorded from pond of Bahona College Campus (Table 1). Chlorophyceae was the dominant group with 24 species and 17 genera. *Closterium*, *Cosmerium*, *Pediastrum*, *Scenedesmus*, *Spirogyra* were found in higher numbers compared to the rest of the genera *Ankistrodesmus*, *Chlamydomonas*, *Chlorella*, *Eudorina*, *Mougeotia*, *Netrium*, *Pandorina*, *Selenastrum*, *Staurastrum*, *Synadra*, *Zygnema*, and *Volvox* which were found to contain a single species each from the study site (Table: 1). Bacillariophyceae was represented by 9 genera and 22 species where major portion of was contributed by *Navicula spp* (8 species) followed by *Pinnularia spp* (3 species). The genera *Cymbella*, *Fragillaria*, *Nitzschia*, and *Stouroneis* contained two species each. On the other hand, *Amphipleura*, *Frustulia* and *Gyrosigma* were represented by single species. Five genera (*Anabaena*, *Microcystis*, *Oscilatoria*, *Phormidium*, *Spirulina*) containing a single species belonging to Cyanophyceae (Myxophyceae) were recorded from the pond. The Euglenophyceae also comprised of almost same number of species (6 species) representing *Phacus*, *Euglena* and *Trachelomonas*. On the other Dinophyceae contained the single species of Genus *Ceratium* (Table 1).

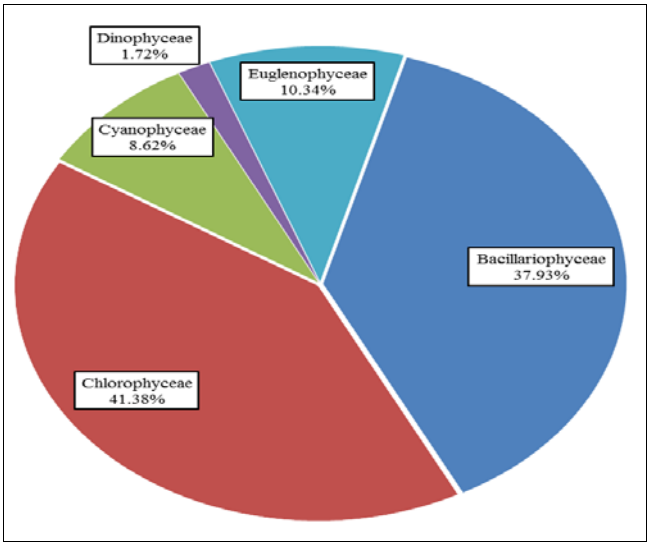

Fig 1: Percentage composition of different Phytoplankton groups

Table 1: Details of Genus and Species belonging to different phytoplankton groups.

Chlorophyceae Total Species: 24 Total Genus:17	*Closterium* (4), *Cosmerium* (2), *Pediastrum* (2), *Scenedesmus* (2), *Spirogyra* (2), *Ankistrodesmus* (1), *Chlamydomonas* (1), *Chlorella* (1), *Eudorina* (1), *Mougeotia* (1), *Netrium* (1), *Pandorina* (1), *Selenastrum* (1), *Staurastrum* (1), *Synadra* (1), *Zygnema* (1), *Volvox* (1);
Bacillariophyceae Total Species: 22 Total Genus: 9	*Navicula* (8), *Pinnuaria* (3), *Cymbella* (2), *Fragillaria* (2), *Nitzschia* (2), *Stouroneis* (2), *Amphipleura* (1), *Frustulia* (1), *Gyrosigma* (1);
Euglenophyceae Total Species: 6 Total Genus: 3	*Phacus* (3), *Euglena* (2), *Trachelomonas* (1);
Cyanophyceae Total Species: 5 Total Genus: 5	*Anabaena* (1), *Microcystis* (1), *Oscilatoria* (1), *Phormidium* (1), *Spirulina* (1);
Dinophyceae Total Species: 1 Total Genus: 1	*Ceratium* (1);

On the other hand, a total of 41 zooplankton species were also recorded from the pond water. The dominance order in terms of number of species was Protozoa > Rotifera > Cladocera > Copepoda > Insecta. The percentage compositions of these groups are shown in Fig 2. The protozoan contribution to the total zooplankton was 13 in which each of genus *Arcella*, *Centrotyxis* and *Paramoecium* added two species each while rest of the other genera *Climacostomum*, *Dileptus*, *Loxodes*, *Pelomyxa*, *Stentor*, *Urostyla* and *Vorticella* have one species each. *Brachionus* was the dominate genus among Rotifera with four species followed by *Keratella* (2 species) and the other Rotifera genera contained one species each (Table: 2). On the other hand, all the seven genus of Cladocera were represented by single species. *Cyclops*, *Mesocyclops*, *Diaptomus*, *Heliodiaptomus* were the four genera under Copepoda in which first two genera contained two species each. Three larval forms of insect *viz*. Crustacean larva, mayfly larva, midge-fly were also recorded during the sampling. (Table: 2)

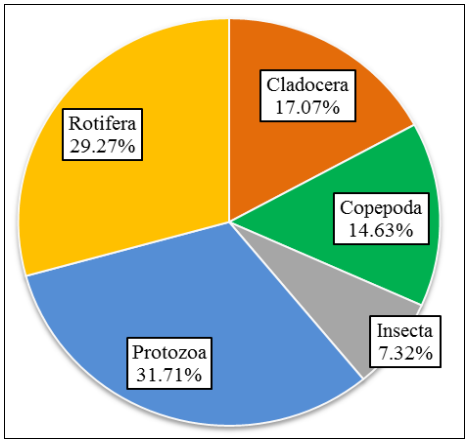

Fig 2: Percentage composition of different Phytoplankton groups

Table 2: Details of genus and species belonging to different Zooplankton groups.

Protozoa Total Genus: 10 Total species: 13	*Arcella* (2), *Centrotyxis* (2), *Paramoecium* (2) *Climacostomum* (1), *Dileptus* (1), *Loxodes* (1), *Pelomyxa* (1), *Stentor* (1), *Urostyla* (1), *Vorticella* (1)
Rotifera Total Genus: 8 Total species: 12	*Brachionus* (4), *Keratella* (2) *Asplanchna* (1) *Collurela* (1) *Conochilus* (1) Eurotatoria (1) *Lecane* (1), *Testudinella* (1)
Cladocera Total Genus: 7 Total species: 7	*Bosmina* (1), *Alona* (1), *Cariodaphnia* (1), *Diaphanosoma* (1), *Daphnia*(1), *Macrothrix*, *Moina* (1),
Copepoda Total Genus: 4 Total species: 6	*Cyclops* (2), *Mesocyclops* (2), *Diaptomus* (1), *Heliodiaptomus* (1),
Insecta Total varieties: 3	Crustacean larva (1), Mayfly larva (1), Midge-fly larva (1)

Discussion

Plankton play key role in the productivity of an aquatic ecosystem. In the present investigation, finding of 99 plankton (58 phytoplankton and 41 zooplankton) species can be considered as significant for a small artificial lentic ecosystem. Among the phytoplankton, Chlorophyceae (41.38%) and Bacillariophyceae (37.93%) were found to be dominant followed by Cyanophyceae (10.34%) and Euglenophyceae (8.62%). Bordoloi and co-workers [5] found

Bacillariophyceae and Chlorophyceae to contribute 35.55% and 32.66% respectively to the phytoplankton population Nahatia wetland of Jorhat District, Assam. In contrast they encountered 31.76% Myxophyceae (Cyanophyceae) in wetland habitat which is greater than present finding. However, they did not considered Euglenophyceae as separate group. Baruah and Kakati[4] reported 45 species of phytoplankton from a pond of ecosystem comprising 16 species Chlorophyceae (35.56%), 14 species of Bacillariophyceae (31.11%), 10 species of Cyanophyceae (22.22%), 3 species of Euglenophyceae (6.67%) and the present study also reflects same pattern of composition. Other recent studies for freshwater lentic water bodies from Brahmaputra and Barak floodplains of Assam [15, 29, 33, 52] and other parts of India also demonstrated similar pattern of phytoplankton composition [3, 47].

The zooplankton population were mainly occupied by Protozoa (31%) and Rotifera (29%) followed by Cladocera (17%) and Copepoda (14%). Three numbers of plankton not belonging to any of these categories are grouped as Insecta category which contributed 7% of the entire record. Consideration of heterotrophic Protozoa as zooplankton have been a matter of choice by researchers as some reports [8, 16, 18] exclude this group of organism from zooplankton whereas many others considered the free swimming forms as plankton [34, 45]. So, irrespective of Protozoan group, the present investigation matches with the findings for freshwater bodies like wetland [18], for pond [10] and reservoir [48]. In contrast, Das and Kar [9] found higher number of Cladocera than that of Rotifera in floodplain wetland of Cachar, Assam. Similarly, Nath *et al*. [33] found dominant number of Protozoan from *Kumri* wetland of lower Assam but opposite to present finding, Copepoda outnumbered Rotifera and Cladocera. Interestingly, Chaudhury found Cladoceran and Rotifera showed almost equal dominance in oxbow lake of Cachar [6]. The community structure of plankton in a water body is determined by various biotic and abiotic factors [32, 49, 50] and such variation may be the result of the influences. The scattered aquatic vegetation in the studied pond along with the lack of major human interference provides ideal microhabitat for phyto and zooplankton enhancing their vertical and horizontal distributions in the pond. Longer studies in relation to season and physic-chemical factors, intervallic diurnal sampling, including some night samples will definitely provide additional information on the structure and dynamics of plankton community in natural as well as artificial aquatic ecosystems.

Report on freshwater Tardigrade

During the present investigation, a plankton sample was found to contain a single unique crawling miniature organism which was later identified as a Tardigrade or Water Bear (Fig. 3). Water bear belongs to a less familiar animal Phylum Tardigrada [37], occupying all possible terrestrial and aquatic habitats [38], but mainly in terrestrial and marine habitats [11, 24]. Tardigrade are considered mysterious animals unlike any other living organism as they can withstand extreme range of temperature [26], have higher tolerance to radiation and toxic chemicals [13], can survive in an elongated anoxic state [7] and even reported to survive in outer space [27] and thus having great scientific importance in understanding biological processes. There are about 1298 species of Tardigrade under three classes - Heterotardigada, Eutardigrada and Mesotardigrada [12]. The information on freshwater Tardigrade is insufficient [22] and the present finding could be helpful in understanding distribution the freshwater group of this animal. Garey *et al*. summarized 62 species in 13 genera as freshwater aquatic Tardigrade representing five families among which only five genera (*Carphania*, *Dactylobiotus*, *Macroversum*, *Pseudobiotus*, and *Thermozodium*) are truly aquatic while the rest eight genera (*Amphibolus*, *Doryphoribius*, *Eohypsibius*, *Hypsibius*, *Isohypsibius*, *Mixibius*, *Murrayon* and *Thulinius*) are limno-terrestrial[23]. In the oriental region, the family Hypsibiidae contains 3 freshwater species along with a doubtful one while there was no information on the genus in the report of Garey *et al*. [23]. Further, most of them are endemic and belong to complex species groups [42] thus making their identification in a new geographical locations challenging.

Tumanov considered the information on the freshwater Tardigrade of India is completely absent [51]. However, Kulkarni and co-workers included a photograph of Tardigrade while studying the fauna of a small temporary pond in Pune, Maharashtra state [28]. But no addition information about this animal was mentioned in the tables or descriptions of the report. Similarly Kumar *et al*. mentioned as '*Tardigrade sp*' under Protozoa while studying two high altitude Himalayan ponds of Uttarakhand [30]. We are not sure whether the authors were indicating Rotifera *Rotaria tardigrada* (Ehrenberg, 1830) or to a Tardigrade specimen. In Northeast India, Dutta and coworkers enlisted a Tardigrade species *Hypsibus* sp. (possibly indicating *Hypsibius*) in the periphyton community of Namsang stream of Arunachal Pradesh [19]. Tumanov provided the first detailed information on the freshwater Tardigrada in India with the finding of species *Pseudobiotus kathmanae* (Hypsibiidae) in a small Himalayan lake [51]. He also discussed the existing taxonomic problem and advocate using genetic analysis and scanning electron micrograph for the taxonomy of Tardigrades.

We have examined several plankton samples from the pond as well as from the surrounding temporal water bodies, but could not find more individuals of it. Several moss samples from the moist twigs and concrete structures, submerged algae and macrophyte leaves were also collected and examined from the vicinity of campus pond but no additional specimen could be recorded. Unfortunately, the pond was drained out for reconstruction purpose before we could do enough sampling to recollect the animal. Neel stated about the irregularity in the occurrence of Tardigrades in a region [36], but with chances of local abundance whereas according to Nelson the freshwater species occupy the benthic zone or crawling on vegetation and sometimes get collected in plankton nets [37]. We expect to find out more specimen by considering wider sampling areas, so that proper taxonomic and molecular examinations can be achieved. Therefore, based on the available information, observations and limitation useful equipments, we are reporting the finding as Tardigrade species of Family Hypsibiidae under Class Eutardigrada of Phylum Tardigrada for the first time from the state Assam but without going further into lower taxonomic ranks. It would be an addition to the limited information on the occurrence of freshwater Tardigrade for India.

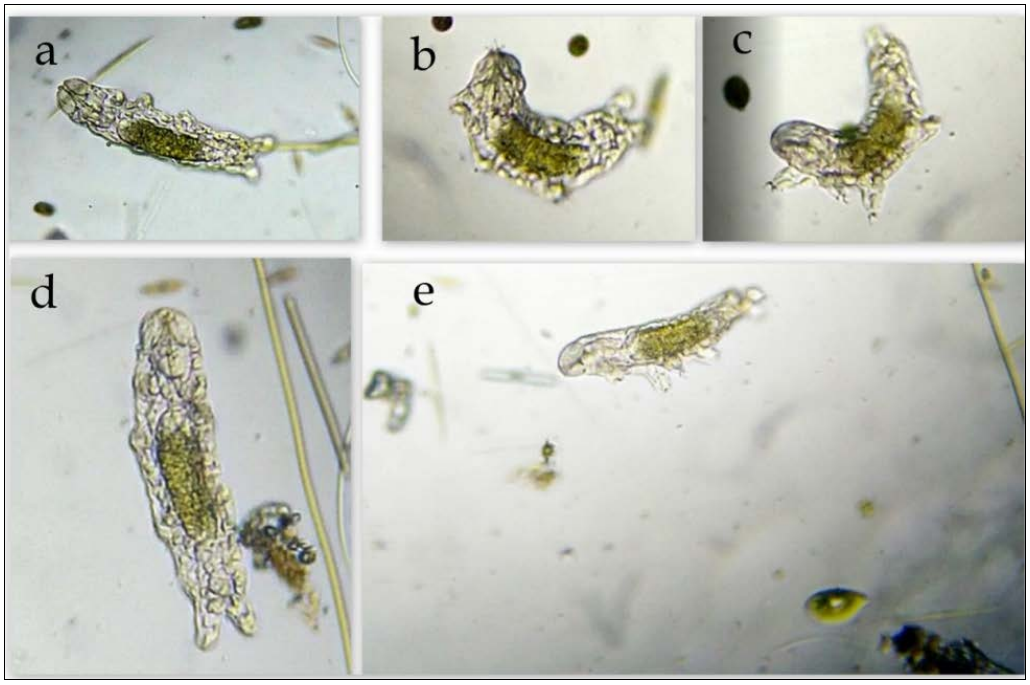

Fig 3: a-d. Ventral and ventro-lateral view of Tardigrade in live condition showing various structures, e. size in comparison to a *Phacus* sp. (green structure lower right corner) (Images captures from a video record of the collected Tardigrade, date: 02-08-2019)

Conclusion

This preliminary study from a small water-body exposed the existence of about 100 species of plankton within a short study period. One of the interesting findings was the presence of freshwater lesser known animal Phylum Tardigrade species which will provide scope for further studies about the zoogeography, taxonomy and ecology of it. Prolonged in-depth study considering the influence of abiotic hydrological parameters will bring out more information in the species assemblage and the interaction of both phyto and zooplankton in such artificial aquatic ecosystems.

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