removing acetonitrile from the reaction mixture, water was added and extracted with ethyl acetate (3 × 20 ml). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, concentrated and separated by silica gel chromatography using gradient mixtures of hexane and ethyl acetate as eluents.

In conclusion a simple, efficient and practical method for direct conversion of piplartine to primary or secondary carboxamides carried out by primary as well as secondary amines under mild conditions has been developed. Studies are in progress in order to investigate the scope of this useful transformation.

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Determinants of 'water fleas' (Crustacea: Branchiopoda: Cladocera) diversity across seasonal and environmental gradients of a polluted river

Cladocera (Crustacea: Branchiopoda), commonly known as water fleas, consist of small, primarily freshwater crustaceans, which form a significant component of zooplankton in different aquatic ecosystems[1]. Currently, about 720 species are known across the globe[2], out of which 130 are reported from Indian waters[3]. Although studies are available on the diversity of Cladocera in the riverine systems focusing on their interactions with the environment and subsequent application as bio-indicators of eutrophication[4–7], relatively less information on their ecology is known from the Indian subcontinent.

Some reliable studies[8–10] are available which document the alpha and beta diversity of Cladocera from the flood-plain lakes in North East India. However, such reliable studies are not common in

Peninsular India and most limnological surveys on the riverine cladoceran diversity of this region are riddled with errors like dubious species identifications[11,12]. Further, to our knowledge, there are no explicit attempts for understanding the diversity of cladocerans in relation to environmental parameters. As a preliminary study, the main purpose of this correspondence is to assess the cladoceran diversity from a lotic water body focusing on correlations of environmental variables with the diversity and the temporal changes observed in the diversity patterns.

Cladoceran diversity was studied at Mula River at Aundh (18.568°N, 73.811°E, 553 m amsl), Pune, Maharashtra, India. Within the city limits, the river is known to be polluted[13]. The breadth of the river at the site is approximately 50 m with depth of about 15–20 m. The site consists of submerged vegetation (*Vallisneria* sp. and *Hydrilla* sp.) seen just after monsoon for a few months and floating vegetation (*Pistia* sp. and *Eichornia* sp.) dominating during winter and summer months. *Lemna* sp. is also seen sporadically along with filamentous algae. The water is turbid (visibility <10 cm) and dark green in colour in summer. The river is fast-flowing during monsoon and slows down temporally with summer season having the least flow. Sewage directly pours into the river, though recently, a sewage treatment plant has been set up near the site. The site is totally inaccessible during monsoon due to the rise in water level. Large number of aquatic insects like Odonate nymphs, dipteran larvae, Heteroptera like notonectids, *Micronecta* sp., *Diplonychus rusticus* and few aquatic beetle species are commonly seen at the site. *Oreochromis mossambicus* and *Gambusia* sp. are observed in high numbers at the collection site.

Sampling was carried out from January 2010 to December 2010, barring July and August due to inaccessibility. Each month, five 1 litre samples were collected from a stretch of approximately 2 m along the bank. The samples pooled together and concentrated in 100 ml cup through filtering in a 100 μm mesh filter. The sampling area was disturbed for 10–15 sec for collecting benthic and epiphytic fauna. Sample was fixed in 5% formalin. Five 1 ml replicates were taken on slides from the 100 ml concentrated sample and observed under stereo microscope for noting the diversity and abundance of cladocerans. Average value of the five replicates was taken and multiplied by the concentration factor (5000/100 ml) and then multiplied by 1000 ml to get individuals/litre estimate[14]. Temperature, pH and salinity values were recorded with a multiparamater probe (Eutech). Type of aquatic vegetation was noted on the field. From the same site, dissolved oxygen (DO) values were obtained every month from the Maharashtra Pollution Control Board (MPCB) website[15]. Average rainfall data for the site were extracted from Worldclime shape file[16] and also used as an indirect measure for water flow.

Relative abundance was calculated first by dividing abundance of each species by the total cladocerans and then dividing it into four categories, viz. rare (R; <5%), average (A; 5–10%), common (C; 10–15%) and abundant (Ab; 15%). Margalef, Shannon–Weiner, Berger–Parker and Evenness indices were calculated to understand the alpha diversity[17] profile of the sample. Bray–Curtis similarity index was used to assess the species complementarity or beta diversity across different months. Canonical correlation analysis (CCorA) was performed to check the multivariate canonical correlations between the multiple environmental variables and multiple alpha diversity indices to understand the effects of environmental parameters on the diversity profile of cladocerans. Diversity indices were calculated using PAST 3.0 (ref. 18) and CCorA was performed in Microsoft Excel ® free addin Biplot 1.1 (ref. 19).

Nine species (Figure 1) from six families were found with Chydoridae being the most species-rich family (Table 1). Previous record[20] of *Simocephalus mixtus* (wrongly identified as *S. vetulus*) and *Karualona* cf. *karua* were not observed

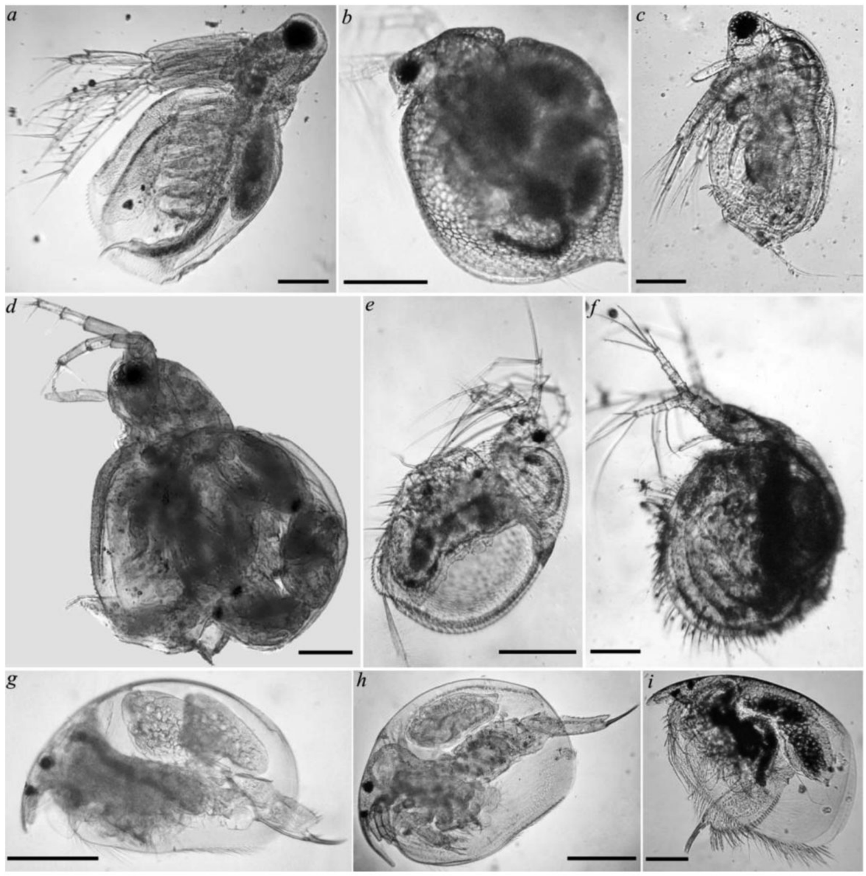

Figure 1. ***a***, *Diaphanosoma sarsi* Richard, 1895; ***b***, *Ceriodaphnia cornuta* Sars, 1885 s.lat.; ***c***, *Moina micrura* Kurz, 1874 s.lat.; ***d***, *Moina macrocopa* (Straus, 1820); ***e***, *Macrothrix spinosa* King, 1853; ***f***, *Ilyocryptus spinifer* Herrick, 1882 s.lat.; ***g***, *Alona cambouei* Guerney et Richard, 1893; ***h***, *Kurzia* (*Rostrokurzia*) *longirostris* (Daday, 1898) and ***i***, *Leydigia* (*Neoleydigia*) *ciliata* Gauthier, 1939 (scale bars represent 100 μm).

Table 1. Species found during different months in the study. Relative abundance categories: R, Rare (<5%); A, Average (5–10%); C, Common (10–15%); and Ab, Abundant (15%)

Species	Relative abundance	Monthly observations January	February	March	April	May	June	September	October	November	December
Diaphanosoma sarsi Richard, 1895	R	+	+	+	+	–	–	–	–	–	+
Ceriodaphnia cornuta Sars, 1885 s.lat.	R	–	+	+	–	–	–	–	–	–	–
Moina macrocopa (Straus, 1820)	A	+	+	+	+	+	+	–	–	–	+
Moina micrura Kurz, 1874 s.lat.	Ab	–	–	–	+	+	+	–	–	–	–
Macrothrix spinosa King, 1853	R	–	–	–	–	–	–	+	+	–	–
Ilyocryptus spinifer Herrick, 1882	R	+	–	–	–	–	–	+	+	+	+
Leydigia (*Neoleydigia*) *ciliata* Gauthier, 1939	R	+	–	–	–	–	–	+	+	+	+
Alona cambouei Guerney et Richard, 1893	A	+	+	+	+	+	+	+	–	–	+
Kurzia (*Rostrokurzia*) *longirostris* (Daday, 1898)	C	+	+	+	+	+	+	–	–	+	+

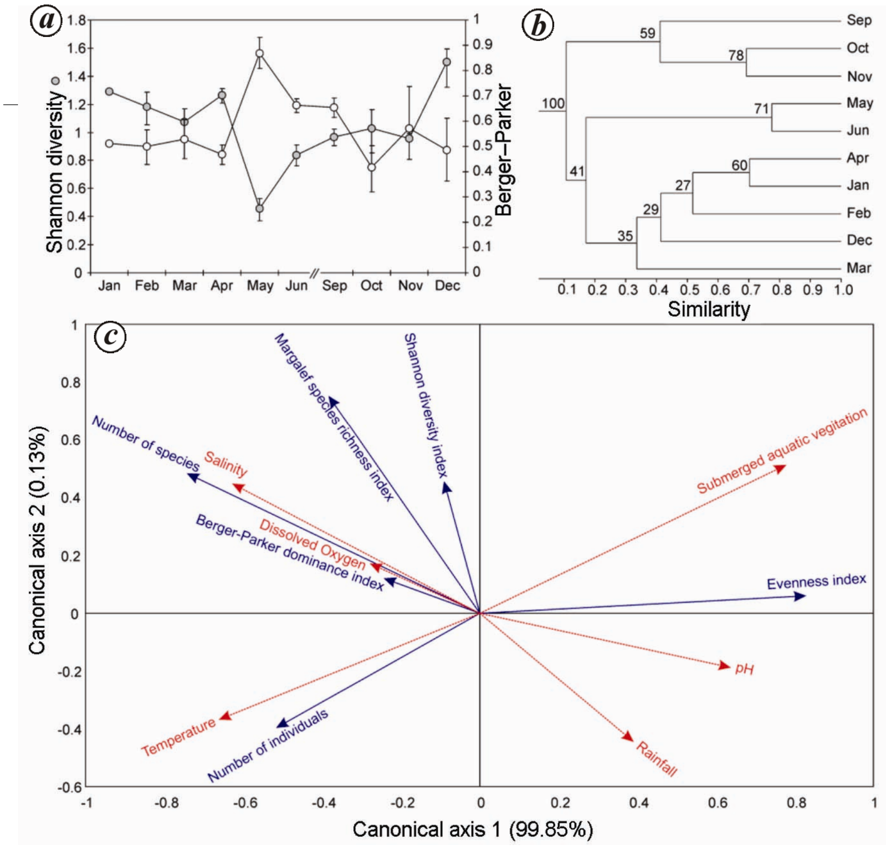

Figure 2. Monthly diversity profile and factors that affect diversity of cladocerans. ***a***, Monthly fluctuations in Shannon diversity and Berger–Parker dominance. ***b***, Bray–Curtis beta diversity across months. ***c***, Relationship between environmental parameters and cladoceran diversity based on canonical correlation analysis. Error bars in (***a***) are standard errors based on 1000 bootstrap iterations. Values along the nodes in (***b***) are per cent bootstrap values for 1000 bootstrap iterations. Values in parenthesis for (***c***) are the per cent variation explained by each canonical axis.

in these collections. Both *Moina* species varied in persistence and abundance with *Moina macrocopa* being more frequent than *M. micrura* s. lat., but less abundant than its congener (Table 1). Maximum species number seen was seven in January (late winter) and least in September (late monsoon) with three species. December (winter) had the highest Shannon diversity value during which six species were observed, while May sample showed lowest Shannon values as it had only four species in which both *Moina* species made up 98% of the total individuals (Figure 2 *a*). Therefore, highest value of Berger–Parker index of dominance was observed in the same month. Lowest Berger–Parker index was observed in October (post-monsoon) when only three species were found and in very low numbers (<50 individuals/l; Figure 2 *a*).

Bray–Curtis-based beta diversity (Figure 2 *b*) showed two distinct clusters, viz. (1) September–November months (late monsoon/early winter) with a good bootstrap support and (2) rest of the months respectively. The second cluster further separated into two groups with a weak bootstrap support: (a) May and June (summer) forming one clade during which *Moina* species dominated, and (b) December–April (winter–early summer) when maximum number of species was observed. *M. spinosa* was observed only in September and October, while *D. sarsi* and *C. cornuta* were seen only during January–April. *M. micrura* was observed only during April–June (summer), while true benthic species like *L. ciliata* and *Ilyocryptus spinifer* did not occur in summer.

The pH of water at the site ranged from 7.12 to 8.05, temperature from 24°C to 31.2°C, salinity from 105 to 386 ppm, DO from 0.81 to 4.15 mg/l, and average rainfall from 0 to 157 mm/year. CCorA of environmental and diversity-related parameters extracted six canonical axes, out of which the first axis explained

99.85% of the total variance while the second axis explained 0.13% of the total variance (Figure 2 *c*). Submerged aquatic vegetation, pH and average rainfall positively correlated with the first axis (0.76, 0.63 and 0.39 respectively), while salinity, DO and temperature correlated negatively with the first axis (–0.61, –0.27 and –0.64 respectively). Barring evenness (0.82), all other diversity indices like Shannon, Margalef and Berger–Parker showed a weak negative correlation with the first axis (–0.09, –0.38 and –0.24 respectively).

In the case of lotic systems, water flow and discharge can alter zooplankton populations, thus affecting their seasonal variations[4,21]. In this study also, it was observed that a change in monthly average rainfall (thus affecting the water flow) influenced species richness and diversity indices negatively (Figure 2 *c*). Highest number of individuals (800/litre) distributed amongst five species was observed in the summer months when temperatures reached >30°C and the site was devoid of any submerged aquatic vegetation with *M. micrura* having more numbers than the rest. This would explain the positive correlation of temperature and negative correlation of submerged aquatic vegetation with both Berger–Parker and Shannon indices. Negative correlation of Margalef and number of individuals with rainfall could be due to the association of rainfall pattern and resulting water flow. Such pattern of high abundance (or biomass) with low rainfall has been reported in other studies[7] and attributed to higher water residence time[5,6]. Negative correlation of DO with rainfall and submerged aquatic vegetation with higher number of taxa cannot be explained and could be a result of small sample size.

A shift in the trophic status of a water body can lead to harmful cascading effect within the plankton communities, thus modifying their organization[22]. Factors like organic pollution of the river along with introduction of exotic fish have already altered the native fish fauna of this river[23]. This is a preliminary time-scale study aimed at elucidating the diversity patterns of Cladocera, and the factors governing their diversity and distribution in the riverine system. Since long term monitoring studies not only highlight the change in the species diversity but also the far-reaching effects of the fluctuations in the diversity caused by environmental perturbations[23], a more detailed survey of Cladocera diversity could yield insights into the ecology of lotic systems. Further, understanding the influence of environmental variables like chlorophyll *a*, primary productivity, phosphorus and nitrate contents, and actual water flow rates on Cladocera diversity could initiate studies on their use as bio-indicators, especially with respect to pollution and eutrophication.

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