Impact of barrages on assemblage pattern of phytoplankton in tropical river Ganga, India

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Abstract The occurrence, abundance, and distribution of phytoplankton have been investigated upstream and downstream of three barrages on the river Ganga at Bijnor, Narora, and Kanpur in Uttar Pradesh, India. A total of 104 phytoplankton species belonging to eight phyla (Bacillariophyta, Charophyta, Chlorophyta, Cryptophyta, Cyanophyta, Euglenophyta, Miozoa, and Ochrophyta) were identified during the sampling period. During the summer, monsoon, and post-monsoon seasons, the density of phytoplankton (Ind. L⁻¹) ranged from 9.6 × 10⁴ to 2.03 × 10⁷, 9.6 × 10⁴ to 4.5 × 10⁵, and 2.2 × 10⁵ to 2.17 × 10⁶, respectively. The species abundance and the relative abundance showed an increasing trend from the first (Bijnor) to the third (Kanpur) barrage, suggesting a gradual decrease in river flow and an increase in residence time. Phytoplankton cell density in Kanpur, however, was unexpectedly higher and showed eutrophic conditions attributable to elevated organic load and surplus nutrients from the land runoff. One-way ANOVA (post-hoc Tukey test) showed statistically significant (p < 0.05) seasonal variation in temperature, transparency, free CO₂, PO₄³⁻, and dissolved organic matter. Analysis of Pearson’s correlation coefficient suggested a statistically significant correlation (p < 0.05) of mostly phytoplanktonic groups with free CO₂, CO₃²⁻, HCO₃⁻, Cl⁻, specific conductivity, total dissolved solids, total hardness, Mg²⁺, PO₄³⁻, and SiO₄⁴⁻. The minimum species diversity was recorded during the monsoon season, while the maximum diversity was reported during the post-monsoon season which might be due to high nutrient load and a high concentration of PO₄³⁻ post-monsoon. We concluded that aquatic biodiversity and ecological structure could be adversely influenced by a series of obstructed barrages and dams, which influenced the assemblage pattern of phytoplankton communities.

Keywords Upstream · Downstream · Ganga · Phytoplankton · Barrages

Introduction

Anthropogenic barriers such as locks, dams, barrages, and weirs drastically minimize the longitudinal connectivity of rivers, impact downstream flow, and
substantially alter the riverine ecosystem. Discharge from dams plays a major role in the management of ecosystems using flow regulation, mixing, nutrient supply, light regimes, and volume of suspended matter. The construction of dams and barrages is not only declining the ecological integrity and water flow of rivers but also affecting the biodiversity of rivers. Hilsa (Tenualosa ilisha) and cowtail stingray (Pastinachus sephens) have collapsed due construction of the Farakka barrage on the river Ganga (Kumar et al., 2022a, b).

Several natural plant and animal communities in the river ecosystem can be used for health assessment and aquatic environment monitoring (Amengual-Morro et al., 2012; Khanna et al., 2007; Matta et al., 2018; Parmar et al., 2016; Pourafraayabi & Ramezanpour, 2014). Phytoplankton are microscopic photosynthetic organisms of plant origin that reproduce quickly in a short period and serve as an excellent indicator of ecological changes in the water besides acting as food for numerous aquatic organisms (Alam et al., 2015, 2020; Surya et al., 2018). In riverine systems, phytoplankton growth is regulated through flow patterns and discharge dilution (Descy et al., 1987; Lack, 1971). Long residence periods and a low dilution process facilitate the proliferation of suspended phytoplankton, leading to nutrient depletion.

Intensive studies on seasonal changes in composition, density, and diversity of phytoplankton in river Ganga and its basins have been carried out (Bantwan, 2018; Jain et al., 2018; Kumar et al., 2020; Matta et al., 2018; Negi et al., 2012). However, studies on assemblage patterns and diversity of phytoplankton upstream and downstream of the barrages on river Ganga are scarce.

In general, a similar pattern of phytoplankton assemblage is noticed in river stretches with the continuous flow till the point there are no obstacles. The present study was carried out upstream and downstream of river Ganga at three barrages. The main aim of this study was to assess seasonal changes and assemblage patterns of phytoplankton along with community structure and to examine the possible impact of the physico-chemical parameters.

Materials and methods

Study sites and sampling designs

This research was conducted during the summer (May), monsoon (August), and post-monsoon (October) seasons of 2016 in the middle stretch of the river Ganga upstream and downstream of Bijnor (lat. 29°22′25″ N, long. 78°02′16″ E, elevation 220 m), Narora (lat. 28°11′28″ N, long. 78°23′55″ E, elevation 178 m), and Kanpur (lat. 26°30′37″ N, long. 80°19′07″ E, elevation 110 m) barrages in Uttar Pradesh (Fig. 1).

Physico-chemical properties

Sub-surface water samples (0.5 m depth) were obtained from three barrages at several sub-locations upstream and downstream and combined to make homogeneous samples. The sub-surface water was collected manually by pre-define volume (5 L) of a plastic bucket at a depth of around 0.5 m in three different sub-locations at the same time by three groups. The collected samples were brought to a place in a short interval of time and mixed together to form homogenous samples. The samples were transferred to a pre-rinsed polyethylene bottle (1.0 L) immediately. With the aid of a multiparameter water monitoring instrument (AQUAREAD AP 2000, manufactured by Aquaread Ltd, Broadstains, United Kingdom), water temperature, conductivity, dissolved oxygen, free CO₂, and pH levels were measured in situ. Transparency was measured using a Secchi disc. Water samples were brought to the laboratory in cold conditions for further analysis of other physico-chemical parameters (e.g., CO₃²⁻, HCO₃⁻, Cl⁻, total dissolved solids, total hardness, Ca²⁺, Mg²⁺, PO₄³⁻, SiO₄⁴⁻, dissolved organic matter following standard methods) (Rice et al., 2017).

Phytoplankton species composition

Homogenous water samples were transferred in one-liter plastic bottles and immediately preserved in Lugol’s solution. The sedimentation method was followed for qualitative and quantitative analysis of the phytoplankton species (Kumar et al., 2015a, b 2017, 2020). The Olympus CX31 trinocular microscope (manufactured by Olympus Corporation, Orinpasu Kabushiki-gaisha, Japan) was used for quantitative analysis (Sedgwick-Rafter cell method), and the phytoplankton were categorized to genus or species level wherever possible based on standard taxonomic identification keys (Bellinger & Sigee, 2010; Cox, 1996; Prescott, 1970; Ward & Whipple, 1992). The known algal taxonomic names were reaffirmed with the AlgaeBase database (Guiry & Guiry, 2020). Phytoplankton were classified according to the AlgaeBase community.
(taxonomic position). The total number of phytoplankton encountered is expressed as Ind. L$^{-1}$.

Statistical analysis

Physico-chemical data were normalized by log($x+1$) transformation except for pH before analysis. Site-wise variation in water quality parameters upstream and downstream was subjected to a one-way analysis of variance (ANOVA) (Fisher, 1925, 1992), and their means were compared through post-hoc Duncan’s multiple range tests (Tavakol et al., 2017) using statistical software, SPSS, ver. 18 (manufactured by IBM and developed by Norman H. Nie, Dale H. Bent & C. Hadlai Hull). Pearson (2-tailed) correlation between parameters of water quality and phytoplankton groups was also computed (Benesty et al., 2009; Bravais, 1844; Freedman et al., 2007). Principal component analysis (PCA) of physico-chemical parameters was performed to evaluate the contribution of major water variables in different sampling stations (Jolliffe, 2002; Pearson, 1901). Diversity indices were calculated following the Shannon-Weiner diversity index (H) (Shannon & Wiener, 1964) and Simpson index of diversity (Simpson, 1949). A graphical representation of the $k$-dominance curve was used to analyze the trend of dominance across seasons (Clarke, 1990). To obtain the degree of similarity between samples/sampling stations, hierarchical cluster analysis was performed and further validated (Nielsen, 2016). Non-metric multidimensional scaling (NMDS) was carried out to investigate the similarity of group community between samples, using measures of similarity ‘Bray–Curtis’ (Cox & Cox, 2001). Biotic (cluster, $k$-dominance, and NMDS) analysis was performed using the Statistical tool Plymouth Routines in Multivariate Ecological Research (PRIMER v 6.1.6) (Clarke & Gorley, 2006).

![Fig. 1 Map showing sampling sites of barrages on river Ganga](https://example.com/fig1.png)
Results

Species composition and abundance

A total of 104 phytoplankton species were recorded during the sampling period under eight phyla, namely Bacillariophyta (52 spp.), Charophyta (6), Chlorophyta (31), Cryptophyta (2), Cyanobacteria (8), Euglenophyta (3), Miozoa (1), and Ochrophyta (1). In both upstream and downstream barrages, seasonal as well as longitudinal variations in species richness and density were observed. Species richness ranged from 14 to 34, 3 to 8, and 21 to 31, while density (Ind.L⁻¹) ranged from 9.6 × 10⁴ to 2.03 × 10⁷, 9.6 × 10⁴ to 4.5 × 10⁵, and 2.2 × 10⁵ to 2.17 × 10⁶ during summer, monsoon, and post-monsoon seasons, respectively.

Species richness was higher in the post-monsoon season, followed by summer, while the overall density of species was highest during the summer season, followed by the post-monsoon season. The lowest density and abundance of phytoplankton were recorded in the monsoon season. From the first barrage (Bijnor) to the third barrage (Kanpur), species richness and density showed an increasing trend, and it was unexpectedly high at the third barrage (Kanpur barrage).

The most dominant group among the phytoplankton was Bacillariophyta, followed by Chlorophyta. The seasonal assemblage of phytoplankton is documented in the present study. Bacillariophyta, Chlorophyta, and Charophyta were recorded in all seasons (Fig. 2). However, Chlorophyta was not recorded downstream of Bijnor and upstream and downstream of Narora during the monsoon season. Cyanophyta and Euglenophyta were not recorded in the monsoon season. Cryptophyta and Ochrophyta were recorded only in the post-monsoon season. Cryptophyta was recorded in Narora and Kanpur barrages, while Ochrophyta was recorded in Kanpur only. The assemblage of phytoplanktonic groups was seen increasing from the first barrage to the third barrage.

Physico-chemical parameters

Seasonal variations of physico-chemical parameters in one-way ANOVA (post-hoc Tukey test) of the three barrages showed significant differences (p < 0.05) for water temperature, transparency, CO₂, PO₄³⁻, and dissolved organic matter (Table 1). However, spatial variations (upstream and downstream) showed no significant differences (p < 0.05) among physico-chemical parameters (Table 2).

Seasonal variations in physico-chemical parameters

Temperature followed a general pattern of weather, and the summer season exhibited the highest water temperature. pH showed healthy with ideal buffer condition of water and was alkaline round the year, and it was ambient value for aquatic life in water. The lowest transparency was recorded during the monsoon season. This is due to high turbidity from monsoon flooding from the catchment.

The highest value of free CO₂ and lowest values of CO₃²⁻ and HCO₃⁻ were recorded during the monsoon season, and this was observed due to the inverse relationship of CO₂ with CO₃²⁻ and HCO₃⁻ by the transformation of one form to another. Dissolved
oxygen was highest in the post-monsoon season, probably due to high photosynthetic activity by phytoplankton and other aquatic plants in water. The lowest values of Cl−, specific conductivity, and total dissolved solids were recorded in the monsoon season. The highest value of total hardness, Ca2+, and Mg2+ was recorded in the monsoon season. Similarly, phosphate was highest in the post-monsoon season. The highest value of SiO4 4− and dissolved organic matter were recorded in the summer season.

Spatial variations in physico-chemical parameters

The mean value of parameters such as temperature, pH, CO3 2−, HCO3 −, specific conductivity, total dissolved solids, total hardness, Ca2+, Mg2+, and PO4 3− showed an increasing trend from the first to the third barrage (Table 2). Other parameters did not show any definite trend; however, their values were unexpectedly high at the third barrage (Kanpur barrage), either upstream or downstream or in both places. Even with the heavy load of pollutants in river Ganga, dissolved oxygen was observed to be ambient for aquatic life at all three sampling places.

PCA biplot analysis of physico-chemical parameters indicated the magnitude of changes in corresponding variables, which increases along the length of the biplot. There were four principal components as HCO3 − (0.37), specific conductivity (0.36), Cl− (0.31), and temperature (0.30) having eigenvalue > 1.00 and which explained 80.72% of the variance. These four components were most heavily weighed on the first component (score > 0.3) and greatly influenced phytoplankton richness and diversity (Fig. 3).

Relationship between physico-chemical properties and phytoplankton communities

The linear association intensity (Pearson’s correlation coefficient) study of phytoplankton groups with physico-chemical parameters revealed essential associations of the phytoplankton in the Ganges. The majority of phytoplanktonic groups in river Ganga are significantly associated (p < 0.05) with free CO2, CO3 2−, HCO3 −, Cl−, specific conductivity, total dissolved solid, total hardness, Mg2+, PO4 3−, and SiO4 4−. A significant positive (Pearson) linear correlation coefficient (r) was observed between Bacillariophyta and CO2 2− (r = 0.507; p < 0.05), HCO3 − (r = 0.691; p < 0.01), Cl− (r = 0.534; p < 0.05), specific conductivity (r = 0.640; p < 0.01), and PO4 3− (r = 0.594; p < 0.01). The association between Charophyta and CO2 (r = 0.510; p < 0.05), total hardness (r = 0.599; p < 0.01), and Mg2+ (r = 0.701;
The upstream and downstream of physico-chemical parameters are not significantly ($p < 0.05$) different from each other. The eigenvalues of the first and second axis explained 80.13% of the variance. The four principal components such as temperature ($-0.88$), HCO$_3^-$ ($-0.56$), Cl$^- (-0.32$), and specific conductivity ($-0.48$) in the CCA triplot weighed heavily on the first axis and were placed left side. Species such as *Tabellaria* sp., *Anomoeoneis* sp., *Gomphonema* sp., *Tetraecynus* sp., *Fragilaria* sp., *Rhicosphenia* sp., *Rhizosolenia* sp., and *Chlorella* sp. exhibited wide tolerance to the four principal components of water quality and were positioned on the right. These species were negatively associated with temperature, HCO$_3^-$, Cl$^-$, and specific conductivity. Similarly, *Synedra ulna*, *Aulacosira granulata*, *Microspora* sp., and *Scenedesmus* sp. have eigenvalue of $>1$ and are placed on the left side of the first axis. These species are positively associated with temperature, HCO$_3^-$, Cl$^-$, and specific conductivity.

CCA analysis between four principal components of physico-chemical parameters and phytoplankton groups was performed to understand the assemblage pattern.
of phytoplanktonic groups in highly variable parameters such as temperature ($-0.16$), $\text{HCO}_3^-$ ($-0.61$), $\text{Cl}^-$ ($-0.24$), and specific conductivity ($-0.42$) (Fig. 5). The eigenvalues of the first and second axis explained 84.52% of the variance. $\text{HCO}_3^-$, $\text{Cl}^-$, and specific conductivity are pollution indicator parameters, and they can be increased in polluted water. Bacillariophyta, Chlorophyta, and Charophyta showed a negative association with these four parameters and were placed right side of the triplot. Negative association indicates the dominance of Bacillariophyta, Chlorophyta, and Charophyta in low pollution water. On the other hand, the left side placement of Cyanophyta, Euglenozoa, Cryptophyta, Miozoa, and Ochrophyta indicated pollution-tolerant species. Miozoa and Ochrophyta showed significant association with polluted water, which were only recorded at Kanpur in the post-monsoon season.

The $K$-dominance plot pattern demonstrated the seasonal dominance of phytoplankton (Fig. 6). Phytoplankton showed greater dominance during monsoon, followed by summer and post-monsoon seasons. However, species abundance did not show many differences during summer and post-monsoon and depicted a similar phytoplankton dominance pattern. The lowest dominance in the post-monsoon season revealed higher abundance and even distribution of phytoplankton species.

Cluster analysis of phytoplanktonic groups for upstream and downstream of three barrages discriminated against two main groups linked to 50% similarity (Fig. 7). The upstream and downstream of the Kanpur barrage formed groups I, with an average similarity of 50.2%. The upstream and downstream of the Bijnor and Narora barrages formed groups II with an average similarity of 45%. High similarities between the two groups were discriminated against by cluster analysis. However, they were indicative of incremental changes in the composition of phytoplankton with barrages.

The ordination of abundance data by NMDS using Bray–Curtis similarity showed that the phytoplankton was generally comparable in Kanpur and Narora barrages with an overall abundance similarity of 20% (Fig. 8). On the other side, between the upstream and
downstream of the Kanpur and Narora barrages, this similarity was very close at about 40%. The similarity of phytoplankton species increased from the first barrage to the third barrage, as shown by NMDS. The stress factor overlying the NMDS plot (0.01) was relatively small, meaning that the river Ganga was not very stressed.

Both Simpson’s diversity index (1-D) and Shannon–Wiener’s diversity index (H) had the highest values in the post-monsoon season and lowest in the monsoon season. Simpson’s diversity index was ranged from 0.9146 to 0.94559, while Shannon–Wiener’s diversity index was ranged from 2.681 to 3.407.

Fig. 4 Canonical correspondence analysis (CCA) of phytoplankton species (>10%) and four principal components of physico-chemical parameters

Fig. 5 Canonical correspondence analysis (CCA) of phytoplankton groups and four principal components of physico-chemical parameters
Discussion

Ecological status of river Ganga

The river Ganga’s natural flow gradually decreased from its origin to the confluence point at the Bay of Bengal due to several anthropogenic barriers such as dams, barrages, and weirs which are installed for diversion and use of river water for industries, agriculture, and domestic purpose. Water quality of the riverine system is significantly degraded by tributaries carrying wastes from many sources, industrial effluents, household waste, and agricultural surface runoff (Herojeet et al., 2017; Kumar et al., 2015a, b, c; Shukla et al., 2018; Wen et al., 2017). Temperature, bicarbonate, specific conductivity, and chloride were the main variables in the current study confirming river water contamination with effluents discharged from industrial sectors, domestic wastes, and agricultural fertilizers and chemicals (Hamid et al., 2020; Khadse et al., 2008; Tiwary et al., 2005). A similar observation was also reported by several researchers at different locations in the river Ganga confirming our finding (Malik et al., 2021; Roy & Shamin, 2020). It was reported that the water quality of the river Ganga had improved during the COVID-19 lockdown due to restrictions on travel and slowing down of industrial operation (Dutta et al., 2020; Muduli et al., 2021; Singh et al., 2022). The increasing trend in the value of physico-chemical parameters from first to third barrages suggests obstructions in the uniform flow of riverine water and increasing residence time. Moreover, significant seasonal differences in important parameters influenced the assemblage pattern of phytoplankton species and communities.

Industrial effluents, agricultural chemicals, and pesticides are sources of chloride ions, specific conductivity, total dissolved solids, and total hardness in water. The lowest values of Cl\(^-\), specific conductivity, and total dissolved solids in the monsoon season might be due to high dilution by monsoonal water and runoff from catchment areas (Kumar et al., 2022a, b). Total hardness was highest in the monsoon season which might be due to the incorporation of turbid water with abundant Ca\(^{2+}\) and Mg\(^{2+}\). Phosphate was highest in the post-monsoon season, possibly due to the enhancement of phytoplankton richness and density. The highest value of SiO\(_4\)^{4-} and dissolved organic matter in the summer season might be due to the persistent release of domestic sewage and a greater decline in the monsoon season due to dilution by monsoonal water.
Kanpur is one of the major industrial cities and is well known for leather, chemical, papers, and other small-scale industries which use water from the river Ganga for their operations and also use as a disposal site for waste materials of industries. Phytoplankton assemblage pattern in varying water quality

Phytoplankton belonging to eight groups displayed a vivid diversity and abundance in the upstream and

**Fig. 7** Hierarchical cluster analysis of the studied stations (KAN UP = Kanpur upstream; KAN DN = Kanpur downstream; NARUP = Narora upstream; NARDN = Narora downstream; BIJUP = Bijnor upstream; BIJDN = Bijnor downstream)

**Fig. 8** NMDS showing the Bray–Curtis similarity patterns of the phytoplankton
downstream in the present study. However, physico-chemical parameters greatly influenced the phytoplankton assemblages’ pattern and diversity. Several authors have also reported similar observations in the river Ganga and other aquatic systems (Dwivedi & Srivastava, 2017; Gogoi et al., 2019; Kumar et al., 2015a, b, 2017, 2020; Matta et al., 2018; Sharma et al., 2016; Vajravelu et al., 2018; Wassie & Melese, 2017). Bacillariophyta, Charophyta, and Chlorophyta were assembled ubiquitously and were suggested to be bearable to high flow and sustained in comparable clean water (Atazadeh et al., 2021). Bacillariophyta was the dominant group in the present study, and several researchers also reported similar remarks on the river Ganga, which is consistent with our findings (Khanna et al., 2012; Kumar et al., 2022a, b; Shukla et al., 2015). Other phyla of phytoplankton are pollution indicator species, and these were also confirmed by the CCA triplot. The absence of Cyanobacteria and Euglena in the monsoon season suggested intolerable high flow and relatively low polluted water due to dilution by precipitation. Cryptophyta and Ochrophyta were recorded in the post-monsoon season in high phosphate, suggesting the pollution tolerance of these phytoplanktonic groups (Kang et al., 2021).

Surface runoff from catchment areas during the monsoon season added nutrients to the water which gradually leached during the post-monsoon season and was utilized by phytoplankton to proliferate. A study of the lower stretch of river Ganga suggested an increase in nutrients in the water column due to the resuspension of benthic sediment during the monsoon season (Kumar et al., 2022a, b). The highest phosphate in the post-monsoon season was evidence of this incident in the present study. The lowest dominance in post-monsoon season in the $K$-dominance plot suggested an even distribution of phytoplankton species.

Similarly, the highest diversity in the post-monsoon season has supported the finding of the high abundance and richness of planktons in the post-monsoon season. Several authors also reported high diversity and evenness in the post-monsoon season in the different aquatic systems at different locations (Guhr et al., 2000; Kumar et al., 2022a, b; Ockenfeld & Guhr, 2003). The low abundance and diversity of phytoplankton in the monsoon season in the current study might be due to the high flow of water current, and only well-adapted phytoplankton species (Cyclotella sp., Navicula sp., Synedra sp.) showed their presence (Inyang & Wang, 2020; Atazadeh et al., 2021). High diversity in the post-monsoon season might occur due to high phosphate in water. The negative association of Tabellaria sp., Anomooneis sp., Gomphonema sp., Tetracyclus sp., Fragilaria sp., Rhicosphenia sp., Rhizosolenia sp., and Chlorella sp. and positive association of Synedra ulna, Aulacoseira granulata, Microspora sp., and Scenedesmus sp. with four principal components in CCA triplot suggesting pollutions from various sources have also influenced the richness and assemblage pattern of species.

Impact of barrages on the ecological functioning of river Ganga

A series of barrages on river Ganga obstructed the water flow and enhanced the pollution by a long period of residence coupled with tributaries effluents. The increasing trend of species richness and density from the first barrage (Bijnor) to the third barrage (Kanpur) in the present study suggests increasing residence time and lower environmental discharge (Matta et al., 2018; Shukla et al., 2015). The cluster and NMDS analyses showed similar trends and the highest phytoplankton abundance upstream and downstream at Kanpur. The higher abundance of phytoplankton downstream indicated lower environmental discharges from barrages during the summer season coupled with nutrients from catchment areas. Kanpur barrage of the river Ganga showed highly disturbed and eutrophic conditions during summer and post-monsoon seasons due to higher residence time, lower environmental flow, and industrial effluents. Environmental flow and ecological status should also be tracked periodically to determine the impact of dams and barrages in the future. Cluster analysis revealed high fragmentation of ecology at Kanpur barrage due to eutrophication by pollution by several sources.

Conclusion

Overall, a rich diversity of phytoplankton was noted in the river Ganga at Bijnor, Narora, and Kanpur barrages. These three barrages control river discharge, and mostly, water is used for irrigation and industrial purposes. Hence, the daily environmental flow must be monitored. The aquatic biodiversity and ecological
structure could be adversely influenced by a series of obstructed barrages and dams, which influenced the assemblage pattern of phytoplankton communities.

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Data availability  The data of the present study are available from ICAR-CIFRI. However, restrictions may be applied to the availability which was used under license for this study. Data can be available from the corresponding author with the permission of the funding agency upon reasonable request.

Declarations

Ethics approval  The authors declare that they have strictly followed all the rules and principles of ethical and professional conduct while completing the research work. No specific permission was required to collect the phytoplankton samples at the study sites. The research activities were carried out following permission from the Institute Research Committee, the approving authority of the institutional research project.

Consent for publication  The authors declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that all have approved the order of authors listed in the manuscript of us.

We understand that the Corresponding Author is the sole contact for the Editorial process. He is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

Conflict of interest  The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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