Spatio-temporal distribution of aquatic biodiversity in Gorai Creek, Sub-Urban Mumbai, India

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ABSTRACT

This study was conducted to assess the faunal diversity in Gorai Creek of Mumbai, India from August 2019 to July 2020. The data collected month wise were pooled together and transformed into three seasons [Monsoon (June to September), Winter (October to February), and Summer (March to May)] and this seasonal data were used for the study of biodiversity pattern. Using the average seasonal species occurrence data as input the following biodiversity indices, S, N, d, J’, H’(loge), 1-Lambda, Delta, Delta*, Delta+, sDelta+, Lambda+, Phi+ & sPhi+ were calculated by using PRIMER (v7.0). It revealed that 90 species of fauna under 70 genera, 50 families, 29 orders, 6 classes and 3 phyla were found to occur in the creek. The order-wise representation of identified fauna was found to be 7 ichthyofaunas, 4 pelecypods, 6 gastropods, 2 cephalopods, 1 shrimp and 1 stomatopod along with 8 avifauna. The present study results indicated that the Gorai Creek ecosystem is endowed with moderate biodiversity that needs to be conserved.

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Introduction

Coastal ecosystems occupy more than 70% of the global water surface area with rich aquatic diversity, abundance and dynamic resource distribution that determines the health and stability of these ecosystems (UNEP, 2006). Estuaries are unique coastal ecosystems utilized by various group of aquatic biota and also man-kind (Qasim, 1973; Ansari et al., 1995; Whitfield, 1999). In recent times, the biodiversity study has been focused to address the issues of declining fauna along with the ecological concerns (Thiel et al., 1995). Claridge et al. (1986) stated that the functionalities of an estuarine creek are the congregation of biota, since it is the place for shelter with rich food; despite fluctuating environmental conditions. The species diversity in estuaries varies with the environmental variables on spatio-temporal scale (Blaber & Blaber, 1980). The monsoon mediated environmental fluctuations in the creek system are the drivers of the spawning and migratory pattern of the aquatic biota (Potts et al., 2015); Nevertheless, the ichthyofaunal exploration (Weinstein & Heck, 1979) with respect to spatial and seasonal variations in species diversity and abundance (James et al., 2008) helps to understand the effect of external perturbations on these coastal ecosystems (Hook, 1991).

With a total area of 1121 ha, Gorai Creek of Mumbai, is one of the important creeks located along the Northwest coast of India, which connects the people socio-culturally through livelihood, transportation, recreation and religious value. A small-scale traditional dol net fishery within the coastal region, is considered to be an economic activity that is predominantly correlated to the rich ichthyofaunal diversity (Sreekanth et al., 2019). The dol net fishery is found to be a significant subsistence activity for the artisanal and motorized fishers in creeks along the Mumbai coast along with the gill net fishery, and hook and line fishery. There are limited studies on phytoplankton, zooplankton, economically important fin fish and shell fish species from this creek. Furthermore, there are no detailed attempts to understand the biodiversity, water and soil quality parameters, and their ecological interactions in this ecosystem.

On the other hand, the creek waters and estuarine complexes are highly prone to diverse anthropogenic factors. These anthropogenic stressors as predicted by the demographic pressure is executed by more than 50% of the population inhabiting coastal areas besides the complications arising from domestic discharges, industrial effluents, indiscriminate fishing, pollution and urban land use pattern (González-Sansón et al., 2022). In order, to characterize fishery, and fish biodiversity in a coastal ecosystem, it is essential to analyze spatio-temporal variation in species diversity, the economic profile of the fishery, ecosystem characteristics, trophic flows and ecological interaction between water-soil parameters and functional fish groups. Thus, the present study was carried out to study the spatio-temporal patterns in biodiversity of the fauna in Gorai Creek along with the ecological interactions using soil and water quality parameters.

Material and Methods

Study Area

In the present study, fish were collected from the five sampling sites [Station 1: Interior of the creek towards freshwater inlet - (19.2311, 72.8186); Station 2: Mid-way near to mangrove areas - (19.2214, 72.8071); Station 3: Near to Essel World- Global Vipassana Pagoda (19.2124, 72.8048); Station 4: Near to off mangrove areas (19.2063, 72.7992); Station 5: Near to Gorai beach (9.1997, 72.7952) towards the mouth of creek] using a dol net from Gorai Creek in Mumbai (Figure 1) from September 2019 to August 2020.

Figure 1. Map showing the study area, Gorai Creek with five selected sampling stations, Mumbai, Maharashtra
Fish Sampling Design and Gear Employed

Sampling was done at each station once in a month by using Dol net. The number of species caught was noted and the collected data were pooled together station-wise as well as season-wise for the calculation of biodiversity indices. Total species occurrence and abundance data were calculated by pooling together the total number of individual species collected from all the five stations during the study period. The period signifies one complete season of dol net fishery status except period of closed season (Monsoon fishing ban) from 10th June to 15th August or Narayali Poornima.

Species Identification

The collected fish samples were sorted and identified up to species level. The collected fishes were washed in clean portable water and preserved in formaldehyde solution for further studies. The preserved fishes were sorted into taxonomic groups and identified at fish biology laboratory of Fish Taxonomy Lab, Fisheries Resource Management, Fisheries Resources, Harvest and Post-Harvest Management Division, ICAR-Central Institute of Fisheries Education, Mumbai. The identification of fishes was carried out with the help of standard literature (Fischer & Bianchi, 1981) and updated with recent literature (Nelson et al., 2016). For Avifauna, bird watching and recording has been carried out with the aid of a binocular and camera with zoom lenses. Recorded birds were identified by using standard literature Ali (1996) and Monga (2003).

Water Quality Analysis

The physicochemical parameters of water such as pH, dissolved oxygen (DO), alkalinity, hardness, total suspended solids (TSS), total dissolved solids (TDS), nitrite, nitrate, phosphate, ammonia, biological oxygen demand (BOD) and chemical oxygen demand (COD) were analyzed as per the standard procedure (APHA, 2012). Surface water temperature was measured using a Celsius mercury thermometer calibrated up to 0.1°C. Water pH was measured on-site by OAKTON eco-tester pH 1 (0.0 to 14.0). The salinity of water for different stations was measured with the help of a handheld refractometer ATAGO S/Mill-E (0-100%).

Biodiversity Assessment

Seasonal and spatio-temporal variability in biodiversity indices such as Shannon-Wiener species diversity (H’), Margalef’s species richness (d), Pielou’s evenness (J’), Taxonomic diversity (Δ), Taxonomic distinctness (Δ*), Average taxonomic distinctness (Δ+), Variation in taxonomic distinctness (Δ+) and total phylogenetic diversity (sPhi+) were calculated using computer software package PRIMER v6.1.9 (Clarke & Warwick, 1998; Clarke & Warwick, 1999; Clarke & Gorley, 2006)

Results

During the study, 90 species of fauna were identified in Gorai Creek, Mumbai under 70 genera, 50 families, 29 orders, six classes and three phyla. The phyla-wise; class-wise; order-wise; and family-wise distributions of recorded species (Figures 2 and 3) indicated the seasonal and spatio-temporal variations (station-wise, season-wise and month-wise) in various biodiversity indices (Tables 1-3). The seasonal and spatio-temporal variations of Shannon-Wiener diversity (H’), were found to be highest at station 5 (3.847) followed by station 4 (3.703) and lowest at station 2 (3.305) and station 1 (3.264). The seasonal values of Shannon-Wiener diversity were found to be in the range of (3.905, 3.636 and 3.530) for the 2019 monsoon, 2020 winter and 2020 summer seasons. The seasonal and spatio-temporal variations of species richness (d) were recorded to be highest at station 5 (16.069), followed by station 4 (15.200) and lowest at station 2 (9.337) and station 1 (8.034). The seasonal variations among the different sampling stations were presented in Table 1. The Pielou’s evenness (J’) index values were found to be highest for monsoon (0.868) and lowest for summer (0.810). During the present study, the spatial variation among the different stations were recorded as, Station 1 > Station 5 > Station 2 > Station 4 > Station 3.

Table 1. Seasonal variations of biodiversity indices

<table>
<thead>
<tr>
<th>Seasons</th>
<th>S</th>
<th>N</th>
<th>d</th>
<th>J’</th>
<th>H’ (loge)</th>
<th>Delta</th>
<th>Delta*</th>
<th>Delta+</th>
<th>sPhi+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monsoon-19</td>
<td>90</td>
<td>3168</td>
<td>19.326</td>
<td>0.868</td>
<td>3.905</td>
<td>91.847</td>
<td>93.932</td>
<td>97.466</td>
<td>5975</td>
</tr>
<tr>
<td>Winter-20</td>
<td>80</td>
<td>1736</td>
<td>17.155</td>
<td>0.830</td>
<td>3.636</td>
<td>94.370</td>
<td>97.567</td>
<td>97.563</td>
<td>5475</td>
</tr>
<tr>
<td>Summer-20</td>
<td>78</td>
<td>1332</td>
<td>16.720</td>
<td>0.810</td>
<td>3.530</td>
<td>93.882</td>
<td>97.747</td>
<td>97.178</td>
<td>5050</td>
</tr>
</tbody>
</table>

Note: S, Total No. of species; N, Total No. of individuals; d, Margalef’s species richness; J’, Pielou’s species evenness; H’, Shannon-Wiener diversity index; Delta, Taxonomic diversity; Delta*, Taxonomic distinctness; Delta+, Average taxonomic distinctness index; sPhi+, Total phylogenetic diversity
Table 2. Spatial variations of biodiversity indices

<table>
<thead>
<tr>
<th>Stations</th>
<th>S</th>
<th>N</th>
<th>d</th>
<th>J’</th>
<th>H'(loge)</th>
<th>Delta</th>
<th>Delta*</th>
<th>Delta+</th>
<th>sPhi+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Station 1</td>
<td>38</td>
<td>615</td>
<td>8.034</td>
<td>0.897</td>
<td>3.264</td>
<td>93.730</td>
<td>97.463</td>
<td>97.048</td>
<td>2750</td>
</tr>
<tr>
<td>Station 2</td>
<td>44</td>
<td>623</td>
<td>9.337</td>
<td>0.873</td>
<td>3.305</td>
<td>92.309</td>
<td>96.324</td>
<td>97.146</td>
<td>3100</td>
</tr>
<tr>
<td>Station 3</td>
<td>55</td>
<td>962</td>
<td>11.726</td>
<td>0.861</td>
<td>3.452</td>
<td>92.111</td>
<td>95.521</td>
<td>97.054</td>
<td>3850</td>
</tr>
<tr>
<td>Station 4</td>
<td>71</td>
<td>1729</td>
<td>15.200</td>
<td>0.869</td>
<td>3.703</td>
<td>93.584</td>
<td>96.527</td>
<td>97.123</td>
<td>4900</td>
</tr>
<tr>
<td>Station 5</td>
<td>75</td>
<td>2308</td>
<td>16.069</td>
<td>0.891</td>
<td>3.847</td>
<td>92.967</td>
<td>94.867</td>
<td>97.108</td>
<td>5000</td>
</tr>
</tbody>
</table>

Note: S, Total No. of species; N, Total No. of individuals; d, Margalef’s species richness; J’, Pielous’s species evenness; H’, Shannon-Wiener diversity index; Delta, Taxonomic diversity; Delta*, Taxonomic distinctness; Delta+, Average taxonomic distinctness index; sPhi+, Total phylogenetic diversity

Table 3. Monthly variations of biodiversity indices

<table>
<thead>
<tr>
<th>Months</th>
<th>S</th>
<th>N</th>
<th>d</th>
<th>J’</th>
<th>H'(loge)</th>
<th>Delta</th>
<th>Delta*</th>
<th>Delta+</th>
<th>sPhi+</th>
</tr>
</thead>
<tbody>
<tr>
<td>August.</td>
<td>81</td>
<td>920</td>
<td>17.372</td>
<td>0.898</td>
<td>3.946</td>
<td>93.861</td>
<td>95.372</td>
<td>97.037</td>
<td>5400</td>
</tr>
<tr>
<td>September.</td>
<td>78</td>
<td>812</td>
<td>16.720</td>
<td>0.907</td>
<td>3.951</td>
<td>94.065</td>
<td>95.522</td>
<td>97.211</td>
<td>5375</td>
</tr>
<tr>
<td>October.</td>
<td>44</td>
<td>499</td>
<td>9.337</td>
<td>0.844</td>
<td>3.194</td>
<td>91.655</td>
<td>96.836</td>
<td>96.459</td>
<td>3125</td>
</tr>
<tr>
<td>November.</td>
<td>41</td>
<td>436</td>
<td>8.686</td>
<td>0.783</td>
<td>2.908</td>
<td>89.202</td>
<td>96.830</td>
<td>96.341</td>
<td>3000</td>
</tr>
<tr>
<td>December.</td>
<td>40</td>
<td>346</td>
<td>8.469</td>
<td>0.841</td>
<td>3.104</td>
<td>91.549</td>
<td>97.455</td>
<td>96.378</td>
<td>2950</td>
</tr>
<tr>
<td>January.</td>
<td>43</td>
<td>264</td>
<td>9.120</td>
<td>0.866</td>
<td>3.257</td>
<td>93.614</td>
<td>97.708</td>
<td>96.872</td>
<td>3225</td>
</tr>
<tr>
<td>February.</td>
<td>33</td>
<td>191</td>
<td>6.949</td>
<td>0.847</td>
<td>2.961</td>
<td>91.386</td>
<td>98.323</td>
<td>97.301</td>
<td>2650</td>
</tr>
<tr>
<td>March.</td>
<td>46</td>
<td>478</td>
<td>9.772</td>
<td>0.822</td>
<td>3.149</td>
<td>92.618</td>
<td>98.301</td>
<td>96.787</td>
<td>3200</td>
</tr>
<tr>
<td>April.</td>
<td>47</td>
<td>381</td>
<td>9.989</td>
<td>0.880</td>
<td>3.388</td>
<td>94.339</td>
<td>97.728</td>
<td>97.109</td>
<td>3275</td>
</tr>
<tr>
<td>May.</td>
<td>55</td>
<td>473</td>
<td>11.726</td>
<td>0.813</td>
<td>3.256</td>
<td>91.337</td>
<td>96.976</td>
<td>97.559</td>
<td>3950</td>
</tr>
<tr>
<td>June.</td>
<td>54</td>
<td>717</td>
<td>11.509</td>
<td>0.802</td>
<td>3.198</td>
<td>84.663</td>
<td>91.254</td>
<td>95.056</td>
<td>3475</td>
</tr>
<tr>
<td>July.</td>
<td>54</td>
<td>719</td>
<td>11.509</td>
<td>0.862</td>
<td>3.437</td>
<td>87.884</td>
<td>91.715</td>
<td>95.318</td>
<td>3550</td>
</tr>
</tbody>
</table>

Note: S, Total No. of species; N, Total No. of individuals; d, Margalef’s species richness; J’, Pielous’s species evenness; H’, Shannon-Wiener diversity index; Delta, Taxonomic diversity; Delta*, Taxonomic distinctness; Delta+, Average taxonomic distinctness index; sPhi+, Total phylogenetic diversity

Figure 2. a. Phyla-wise, b. Class-wise representation of species from Gorai Creek, Mumbai
The estimated taxonomic diversity ($\Delta$) values for the sampling stations were recorded highest in winter (94.370) followed by summer (93.882) and lowest in monsoon (91.847). The seasonal variation of estimated taxonomic diversity values was found to be in the descending order as, winter (94.370) > summer (93.882) > monsoon (91.847). The seasonal variation in taxonomic distinctness was observed to be in the range of 93.932 to 97.747. Among the five sampling stations studied, the taxonomic distinctness ($\Delta^*$) was found to be the lowest (94.867) in station 5 and the highest (97.463) in station 1. The overall trend indicated lower average taxonomic distinctness during the monsoon period with increment in winter and highest in
the summer period. The seasonal variation in taxonomic distinctness ($\Delta^+$) among the studied sampling stations was recorded in the order of winter (97.563) > monsoon (97.466) > summer (97.178). The spatial variation in taxonomic distinctness among the studied sampling stations (Table 3) was in the following descending order, station 2 (97.146) > station 4 (97.123) > station 5 (97.108) > station 3 (97.054) > station 1 (97.048).

The total phylogenetic diversity ($s\Phi^+$) value for station 1 is 2750, station 2 (3100) and station 3 (3850), station 4 (4900) and station 5 (5000). The seasonal variation in total phylogenetic diversity ranged from 5475 to 5050. The results of the K-dominance curve were obtained by plotting percentage of cumulative abundance against species rank K on a logarithmic scale. Among the sampling stations, station 5 and station 4 indicated relatively larger cumulative abundance than the other station waters. As the percentage contribution of each species was added, that curve extended horizontally before reaching the cumulative 100%. The result from the K-dominance curve was plotted season-wise. Cumulative relative abundances were higher in monsoon followed by winter and summer. The month-wise D dominance plot showed that the cumulative abundance was rich during August, 2019 and September, 2019 and poor during February, 2020 (Figure 4). The spatio-temporal variation in the K dominance plot among the sampling stations indicated that the flow of cumulative abundances is the flow of station 1>2>3>4>5 (Figure 5). The seasonal variation recorded for Gorai Creek resulted in high during monsoon, 2019 and less during summer, 2020 (Figure 6).

Bray-Curtis similarity is useful in quantifying the compositional similarity between the stations (Figure 7) and seasons (Figure 8). The seasonal variations revealed that the winter and summer seasons have formed clusters together and the monsoon season forms separate clusters for the study area. The seasonal variability in the BC similarity index for individual sampling stations was found to be higher (66.55%) between station 4 and station 5, and 63.13% between station 2 and station 3. Contrary to this, the lowest similarity (23.24%) was observed between station 1 and station 5, followed by 28.50% of station 2 and station 5.

The data collected were pooled for the normal probability test (PAST v3.1.1) and found to be a normal distribution of Aves (0.9237), Actinopterygii (0.9739), Bivalvia (0.9708), Gastropoda (0.9857), Malacostraca (0.8953) and Cephalopoda (0.8995), presented in Figure 9. The correlation matrix reveals (SPSS v22.0) that there are significant differences observed among the environmental variables and the species recorded. The Pearson’s correlation showed that there is a positive relation between DO and pH (0.963**), nitrite and water temperature (0.916) & alkalinity (0.960**), alkalinity (0.986**) & nitrite (0.969**); Soil pH and Bivalvia (0.955*), Gastropoda (0.972**) in which is represented in PCA Plot (Figure 10) with factor loadings plot for the corresponding eigen values and in Table 5 (*Correlation is significant at the 0.05 level, 2-tailed; **Correlation is significant at the 0.01 level, 2-tailed).

Figure 5. K-dominance plot among different spatio-temporal variations at Gorai Creek, Mumbai
Discussion

A total of 15 species of avian fauna belong to 8 orders, 11 families were recorded from the Gorai Creek, all are those species often hovered in coastal wetland areas. Still, the diversity shows more diminutive than the avian fauna recorded by Lad & Patil (2014) recorded 131 avian species belonging to 45 families including resident and migratory birds from Bhayander and Naigaon, Maharashtra. Out of 45 families observed in the study area, family Scolopacidae and Accipitridae represented 12 species each followed by Ardeidae and Sylviidae with 8 and 7 species, respectively while family Laridae, Motacillidae and Rostratulidae with 6 species each. It was seen that, 68% of the species were resident while 32% were migratory. However, the present study was comparatively less to the avian species recorded along with the wetland areas of Gorai Creek. The reason attributed for low diversity of avifauna is attributed due to the loss of habitat by reclamation of land for
Figure 8. Dendrogram showing similarities between seasonal variations based on the composition of species identified in Gorai Creek, Mumbai.

Figure 9. Correlation matrix for the species recorded and environmental variables.
the construction of residential complexes and the reduction of nesting sites (Lad & Patil, 2014). Chauhan et al. (2008) documented the avian fauna in the Gorai proximity and recorded 66 species. Based on the category made by Chauhan et al. (2008) the current study revealed that three species are migrant and uncommon, one species is resident and uncommon, and one species is migrant and uncommon. Nevertheless, the diversity is more compared to the findings of Sinnarkar et al. (2013) conducted in Mahim Bay, Mumbai.

On a global and Indian scale, a lot of studies have been undertaken to file the fish diversity, fishery and their spatio-temporal patterns in various coastal ecosystems (Tremain & Adams, 1995; Ansari et al., 1995). The ichthyofauna has been studied by several marine biologists and fisheries professionals along the Indian coast who documented 603 species from the Laccadive archipelago by Jones & Kumaran (1980), and 1367 species by Venkataraman & Wafar (2005) along the Indian coast.

The present study reveals the availability of 35 fish species, 5 bivalves, 15 gastropods, 2 cephalopods, and 18 malacostraca. The recorded diversity is comparatively less than the other creek ecosystem of Maharashtra reported by Lad & Patil (2013) and Garima et al. (2020). In the present study, the ichthyofaunal diversity indices were optimum, but the number of recorded species was less. The highest ichthyo diversity and evenness occurred in station 5 during the monsoon season, which may be due to less pollution pressure compared with the other sites and the availability of nutrients during the rainy season. Perciformes and decapods contribute the maximum catch. Station 1 is located near the mouth of Gorai Creek adjacent to the sea, which could expect more diversity. The economic activities like jetty construction, fishing harbour and pollution discharged by the tourist from the adjoining beach causes a substantial negative impact on the site. Garima et al. (2020) studied the ichthyofauna diversity of Karanja and Dharmatar creek which uncovered the effect of anthropogenic activities on the studied site.

Lad & Patil (2013, 2016a, 2016b) recorded 53 species belonging to 23 families and 6 orders. The study also reveals that the fish diversity along the estuarine area of Bhayander and Naigaon was satisfactory in comparison to the fish diversity of other estuaries. The fish diversity offers good support to the livelihood of fishermen residing in the adjoining villages. But, the mangrove forest ecosystem of the various estuaries is under threat due to various anthropogenic activities and it is necessary to take some constructive steps to maintain the mangrove ecosystem that indirectly helps in the maintenance of fish diversity. Lad & Patil (2016a) recorded 23 species of meiobenthos belonging to 8 phyla from Bhayander and Naigaon. The influence of environmental variables on fish diversity, assemblages, feeding and breeding grounds was reported by numerous researchers (Madhupratap et al., 2001;
Janureguizar, 2004; Shirodkar et al., 2012). The monsoon, winter and summer seasons are characterized by unique changes in the wind pattern, current shifts, water turbulence, river discharges and temperature along the coastal regions (Madhupratap et al., 2001; Shirodkar et al., 2012). Similar to the observations made by Qasim & Sen Gupta (1981) this ecosystem also experiences seasonal fluctuations in water quality parameters. In the purview of Ansari et al. (1995) and Shamsan (2008), the fish species in the mangrove-associated estuary, have their own strategy for breeding, larval development, and feeding migrations to match the environmental situation. Hence, many finfish and shellfish species are highly dependent on estuarine ecosystems for completing their life history and survival. The current study highlighted the spatio-temporal variation of fish assemblages assessed by biodiversity indices and correlated with environmental variables of the tropical coastal creek ecosystem. The reports by Ansari et al. (1995) and Shamsan (2008) revealed that, the estuaries are endowed with rich aquatic biodiversity and species assemblages. Yet, Gorai Creek is found to be moderate in the line of research, hindered by anthropogenic activities and plastic pollution. Therefore, Gorai Creek plays an important role in the conservation and replenishment of aquatic life and a suitable practical fisheries management strategy is required for the sustainable fisheries management of the creek. Community and co-management practices with proper monitoring of fish catch, reporting systems, fishing holidays, seasonal and regional closures could be considered the practical solution for the sustainable utilization of the resources (Sreekanth et al., 2018).

Conclusion

The present study indicates that Gorai Creek is endowed with moderate biodiversity of flora and fauna. The water and soil quality parameters revealed that the health condition is impacted by the non-biodegradable debris and domestic sewage pollution. The creek ecosystem and its associated biodiversity are subjected to various anthropogenic stressors on a wide range in Mumbai, Maharashtra. Though the study interprets the biological aspect, the data generated could be used for framing the mixed fisheries management as ecological indicators which encompass biological, physio-chemical and socio-economic dimensions for resolving the fisheries complexities in the tropical multispecies paradigm. The study also recommends the valuation of the essential economic benefits of the creek could reveal the exact efficiency and prompt measures for formulating policies and implementing rules and regulations.

Acknowledgements

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Compliance With Ethical Standards

Authors’ Contributions

Author AKJ designed the study, SC wrote the first draft of the manuscript, SBS has done literature analyses, SGB performed and managed statistical analyses, GD and BBN corrected the draft. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References


