

Effects of abiotic changes on a pelagic Chaetognath *Zonosagitta bedoti* population of the Ganges Estuary, India

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ABSTRACT

Chaetognaths form a large biomass and are benthic-pelagic links in tropical river-estuaries. Abiotic variability of the Ganges estuary does change within short temporal intervals. The population abundance of *Zonosagitta bedoti* and the salinity, water temperature and pH variability of the Ganges estuary were examined at 12-hours (hourly interval), 24-hours (hourly interval) and seasonal (at least 3 samples per season) scales. The objective of this study was to evaluate if the associations between population abundance and abiotic variability of the Ganges estuary remain consistent even if the temporal scale of the sampling changes. *Zonosagitta bedoti* population abundance did not vary significantly with 12-hours, 24-hours and seasonal cycles. Associations of *Z. bedoti* abundance with water temperature and pH of the Ganges estuary were not significant in the 12-hours, 24-hours and seasonal studies. In the 12-hours study, *Z. bedoti* abundance showed a significant positive association with salinity; however, such was not observed in the 24-hours and in the seasonal studies. Results suggested that the associations of *Z. bedoti* population abundance with abiotic variables of the Ganges estuary may not always necessarily depend on the temporal scale of the sampling. Such an insight may help to conceptualize a cost-effective ecological monitoring of the pelagic chaetognaths of tropical river-estuaries.

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1. Introduction

A 'one size fits all' temporal scale of studying the association of a population parameter of a species and an abiotic variable of an estuary hardly exists (Paul and Calliari, 2019). River-estuaries are productive ecotones between rivers and seas (Whitfield, 1992); however, a global definition of an estuary is yet to be established (Paul *et al.*, 2019). The salinity levels of river-estuaries vary with tidal and seasonal changes, and the pH levels are closely correlated with the change in salinity; therefore, the changes in salinity and pH of an estuary need to be monitored with high-frequency sampling (Chew *et al.*, 2015; Paul *et al.*, 2019). The water temperature of a river-estuary may or may not change on an hourly, tidal and daily basis, but seasonal change in water temperature is often marked so that it could be studied less frequently than the salinity and pH of an estuary (Litaker *et al.*, 1987; Livingstone, 1987; Paul *et al.*, 2019). The abiotic environment of an estuary affects its inhabitants, but some cope with the abiotic changes by habitat-specific adaptations (Costa *et al.*, 2008; Araujo *et al.*, 2017; Nandy and Mandal, 2020). A large number of estuarine studies have focused on monthly to seasonal sampling for assessing the habitat-specific adaptations of the plankton community (Costa *et al.*, 2008; Paul *et al.*, 2016; Nandy and Mandal, 2020) but the effects of tidal and daily changes of the abiotic environment on plankton are less understood (Chew *et al.*, 2015; Paul *et al.*, 2019).

In the tropical river-estuaries chaetognaths are often the second most dominant zooplankton after the copepods (Srinivasan, 1971; Nair and Selvakumar, 1979; Coston-Clements *et al.*, 2009; Bhattacharya *et al.*, 2015; Chakraborty *et al.* 2022). Further, they are the predators that mainly prey on copepods and; therefore, play an effective role in energy

transfer to higher trophic levels of estuarine food chains (George, 1952; Srinivasan, 1971; Nair and Selvakumar, 1979; Nair *et al.*, 1992). High salinity gradients often control the occurrence of the chaetognaths within a tropical river-estuary (Bhunia and Choudhury, 1983; Nair *et al.*, 1992); therefore, chaetognaths remain generally high near the estuary mouth (George, 1952; Sarkar *et al.*, 1986; Li *et al.*, 2009). The chaetognath *Zonosagitta bedoti* (average adult body length is 15 mm) is an epipelagic, semi-transparent member of the holoplankton of river-estuaries and open waters of the Indo-Pacific between 40°N and 40°S and it preys on copepods, mainly nocturnally (George, 1952; Ghirardelli, 1969; Ball and Miller, 2006; Coston-Clements *et al.*, 2009; Karati *et al.*, 2019). *Zonosagitta bedoti* and *Flaccisagitta enflata* (previously known as *Sagitta enflata*) are the two chaetognaths that are common in the lower stretches of the Ganges estuary (GE) of India, and together they constitute a considerable planktonic biomass in the Indian Sundarbans (Bhunia and Choudhury, 1983; Sarkar *et al.*, 1986; Bhattacharya *et al.*, 2015; Nandy *et al.*, 2018; Nandy and Mandal, 2020; Chakraborty *et al.* 2022). Little is known, however, about the associations of *Z. bedoti* population abundance and abiotic variables of the GE. The effects of salinity, water temperature and pH variability on the *Z. bedoti* population abundance were studied by conducting a 12 hours, a 24 hours and a seasonal studies in the GE with the hypothesis that the associations between population abundance and variability of the salinity, water-temperature and pH would not depend on the temporal sampling scale. This may help in developing a cost-effective way of ecological monitoring of the pelagic chaetognath populations of the tropical river-estuaries.

2. Materials and Methods

2.1. Study site

The Ganges estuary is a large macrotidal (tidal amplitude > 5 m) funnel-shaped tropical river-estuary, which experiences fluctuations in its abiotic environment with tidal and seasonal changes (Mukhopadhyay *et al.*, 2006; Choudhury *et al.*, 2015). The study was conducted firstly in the Muriganga River (MR) and secondly in the western channel of the Saptamukhi River (SR), both the rivers are the part of the GE (see Fig.1). They have moderate mangrove patches that include *Avicennia officinalis*, *A. alba*, *A. marina*, *Aegiceras* sp., and *Excoecaria agallocha*. Both the MR and the SR are in the Indian Sundarbans which has three distinct seasons (Bhattacharya *et al.* 2015): a hot humid pre-monsoon (PRM) from late February to the middle of June, a warm, humid monsoon (MON) between late June and September (Up to 74% of the total precipitation occurs during the monsoon with an average annual rainfall of 150–200 cm), and a moderately cool post-monsoon (POM) season between October and early February (Bhattacharya *et al.*, 2015; Nandy *et al.*, 2018; Chakraborty *et al.*, 2022).

2.2. Sampling for the 12-hours, 24-hours and seasonal studies

On the MR a 12-hour and a seasonal study were conducted, for which a total of 3 sampling sites M1 (21° 44'53.8"N; 88°12'46.2"E), M2 (21°44'55.7"N; 88°12'40.0"E) and M3 (21°44'55.4"N; 88° 12'36.8"E) (Fig.1). Conducted on 24 February 2019, the 12-hours study (hourly interval) had a daylight collection (1–5 p.m.) and a collection after sunset (6.00

p.m.–12.00 a.m.). The seasonal study was conducted on various occasions (at least 3 samples per season) between February 2019 and January 2021.

In the MR, permission for a continuous 24-hours study (hourly interval) was not granted by the local authority, so the study was conducted on the western branch of the SR stretch of the GE on 15 to 16 January 2020 from 3 sampling sites along the north-south direction (Fig. 1). Those sites were S1 (21°45'50" N; 88°16'59" E), S2 (21°45'66" N; 88°16'54" E) and S3 (21°46'05" N; 88° 16'48" E). Samples collected during the 24-hours study were split into two large categories: daylight collections (9.05 a.m.–17.30 p.m. on 15 January 2020 and 5.55 a.m. to 8.50 a.m. on 16 January 2020), and collections after sunset (17.50–23.50 p.m. on 15 January 2020 and 24.05–5.35 a.m. on 16 January 2020). The 24-hours study spanned a full tidal cycle of the GE.

2.3. *Zonosagitta bedoti* collection and laboratory analysis

On each sampling occasion, 100 L of estuarine water was collected using a 10 L plastic bucket and filtered through a 200 µm mesh. Samples were preserved immediately in 4% buffered formalin solution and delivered to the laboratory. When processing, multiple 1 ml aliquots were prepared from the total filtered sample and examined under a stereo microscope (Bestscope-BS30T, China) using a Sedgwick Rafter scale. *Zonosagitta bedoti* individuals were identified (no attempt was made to identify different life history stages) using Nair *et al.* (1992) and their abundances were expressed as individual per cubic metre (ind.m⁻³).

2.4. Measurement of the abiotic factors

For the 12-hours, 24-hours and seasonal studies, salinity (as Practical Salinity Units), water temperature (°C), and pH levels at the sampling sites were measured by using a hand-held multiparameter probe (YSI-1030, USA) from the sub-surface water.

2.5. Statistical analysis

CRAN-R 4.0.3 was used for all the data analysis and α was 0.05 level. In the 12-hours study, salinity, water temperature and pH data were non-parametric so the spatial variations were assessed by the Kruskal-Wallis tests and the temporal variations were examined by the multiple generalized linear models (GLM) of the lognormal family. In the 24-hours study, the pH and water temperature data were non-parametric, but the salinity data showed a parametric distribution. So, the spatial variability of the pH and water temperature was analyzed using the Kruskal-Wallis tests, whereas the spatial variability of salinity was analyzed through an ANOVA. For the 24-hours study, temporal variations of the pH and water temperature were assessed by the multiple GLM of the lognormal family. For an assessment of the temporal variability of salinity, a GLM of the Gaussian family was used. For the seasonal study, salinity, water temperature and pH data showed non-parametric distributions so multiple Kruskal-Wallis tests were conducted to evaluate the spatial variability. Multiple GLM of the lognormal family were conducted to evaluate the temporal variability. In all the cases, if an ANOVA or a Kruskal-Wallis test was found to be significant, then either a Tukey's post-hoc t-test or a Nemenyi post-hoc test was conducted to evaluate the site-specific variations. For the assessments of the hourly variability of the *Z. bedoti*

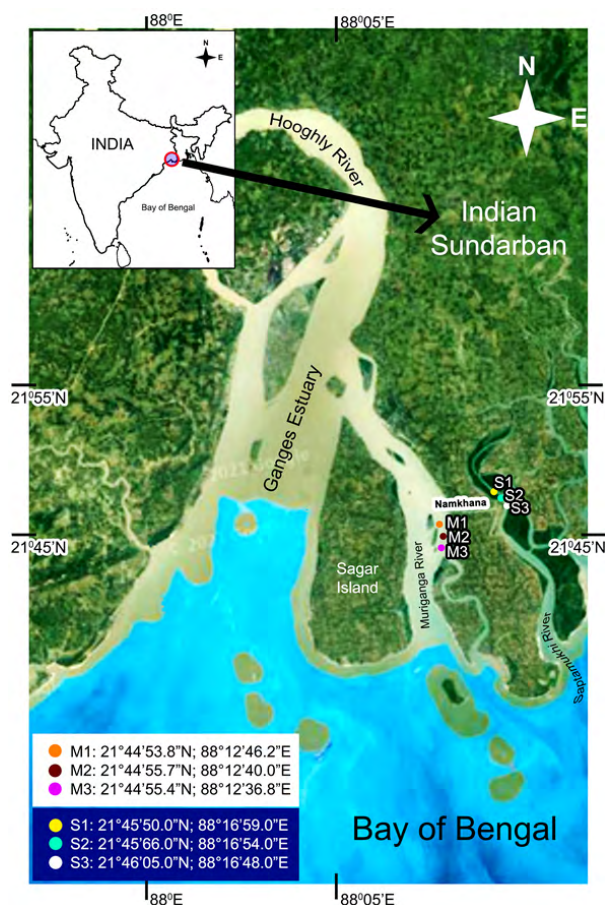


Fig. 1. Sampling sites on the Muriganga (M1, M2, M3) and the Saptamukhi (S1, S2, S3) rivers of the Ganges estuary

population abundance (applicable to the 12-hour and 24-hour studies), multiple GLM of the quasi-Poisson family were used because the abundance data were rightly skewed and over-dispersed. Seasonal variability (among PRM, MON and POM) of the *Z. bedoti* population abundance on the MR was analyzed by using a Kruskal-Wallis test. In the case of 12-hours, 24-hours and seasonal studies, associations of the *Z. bedoti* population abundances and the salinity, water temperature and pH levels of the GE were analyzed by using multiple GLM of the quasi-Poisson family.

3. Results

3.1. 12-hour study

Salinity (median 9.57 ± 0.33 (SE)) did not show any significant spatial variation. The highest salinity (13.60) was recorded at hour 12 at M1 and M3, and the lowest salinity (7.50) recorded at hour 3 at M2 and at hour 9 at M3 (Fig. 2a). Salinity did not vary between the day and night hours. The water temperature (median $24.05 \pm 0.19^\circ\text{C}$ (SE)) did not show any significant spatial variation among the sampling sites. The highest water temperature (26.45°C) was recorded at hour 1 at M1 and the lowest (23.03°C) was recorded at hour 6 at M1 (Fig. 2b). The water temperature (day time median $25.70 \pm 0.23^\circ\text{C}$ (SE)) significantly declined ($t = -8.79$, $DF=35$, $P < 0.01$) during the night (median $23.90 \pm 0.08^\circ\text{C}$ (SE)). The pH level (median 7.40 ± 0.06 (SE)) hardly varied between the sampling sites. The highest pH level (8.57) was recorded at hour 1 at M1 and the lowest (7.03) at hour 11 at M3 (Fig. 2c). The pH level did not significantly decline ($t = -0.45$, $DF = 35$, $P = 0.65$) during the course of the study. The abundance (median 705 ± 126 ind.m⁻³ (SE)) of *Z. bedoti* did not significantly vary during the 12-hour study. The highest *Z. bedoti* (median abundance 990 ind.m⁻³) number of individuals was collected at hour 4 of sampling (Fig. 2d). No *Z. bedoti* individuals were collected at hours 5 and 9 (Fig.2d). *Zonosagitta bedoti* abundance showed a significant positive association ($t = 2.08$, $DF = 35$, $P = 0.045$) with the salinity level of the MR, but no such significant temporal associations were found with water temperature and pH (Table 1).

3.2. 24-hour study

Spatial variation of the salinity (19.88 ± 0.50 (SD)) was not significant; however, the day vs. night variation was significant ($t = 3.96$, $DF=71$, $P=0.0001$). The highest salinity (21.20) was measured at hour 23 at S3 and the lowest (18.80) at hour 15 at S2 (Fig. 3a). Water temperature ($21.70 \pm 0.05^\circ\text{C}$ (SE)) and pH (8.14 ± 0.01 (SE)) did not vary spatially. The water temperature dropped significantly at night ($t = -2.76$, $DF=71$, $P=0.007$) compared to the day time; the highest water temperature (22.60°C) was recorded at hours 6 and 7 of sampling and the lowest (19.30°C) recorded at hour 5 at the S3 (Fig. 3b). The pH did not show significant variation between the day and the night hours ($t = 0.97$, $DF = 32$, $P=0.34$). The highest pH (8.38) was measured at hour 22 at the S2 and the lowest (7.98) measured at hour 1 at the S1 and the S2 (Fig. 3c). No significant spatial variation of *Z. bedoti* abundance was observed. Also, there was not any significant day vs. night

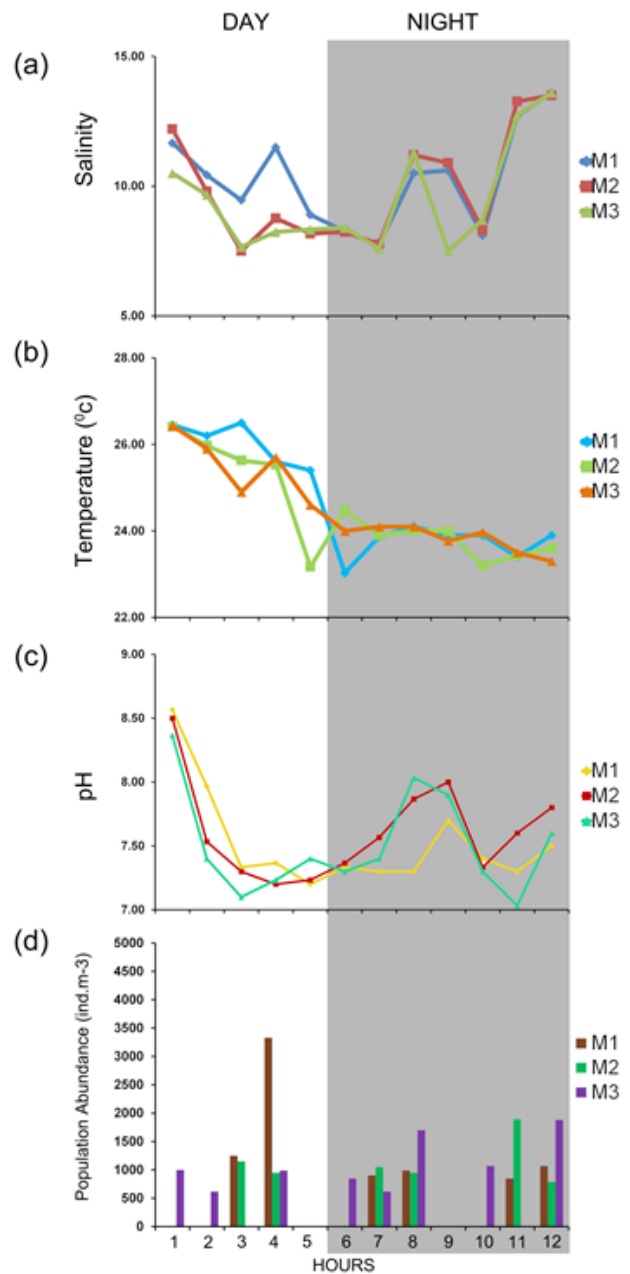


Fig. 1. 12-hour variability of salinity, water temperature ($^\circ\text{C}$), pH and *Zonosagitta bedoti* (ind.m⁻³) abundance in the Muriganga river stretch of the Ganges estuary.

Table 1. Associations of *Zonosagitta bedoti* abundance (ind.m⁻³) with salinity, water temperature ($^\circ\text{C}$) and pH levels of the Ganges estuary, India, at different scales of temporal sampling.

Sampling scale	Abiotic variables	t value	DF	P value
12-hours (hourly interval)	Salinity	2.08	35	0.04
	Water temperature	0.13	35	0.90
	pH	0.44	35	0.66
24-hours (hourly interval)	Salinity	0.14	71	0.89
	Water temperature	0.45	71	0.65
Seasonal (minimum 3 samples/season)	pH	-0.54	71	0.59
	Salinity	0.70	32	0.49
	Water temperature	0.31	32	0.76
	pH	0.66	32	0.51

variation of *Z. bedoti* abundance. The highest *Z. bedoti* (median abundance 4000 ind.m⁻³) number of individuals were collected at hour 22 of sampling (Fig.3d). Irrespective of the sites, samples collected at 9, 10, 12, 17 to 20, and 24 hours had no *Z. bedoti* individuals (Fig.3d). Temporal changes of *Z. bedoti* abundance were not significantly related to the salinity, water temperature and pH levels of the SR (Table 1).

3.3. Seasonal study

Salinity (median 10.90 ± 0.84 (SE)) did not show any significant spatial variation. The highest salinity (19.30) was observed in August 2019 at the M1 and the M3. The lowest salinity (4.80) was observed in June 2020 at the M2 (Fig. 4a). Water temperature (median 27.27 ± 0.64°C (SE)) also showed no significant spatial variation; however, its temporal variation was significant (K-W chi-square =9.31, DF =2, P=0.009) among PRM (February-May) (median 29.95 ± 0.95°C (SE)), MON (June-September) (median 23.20°C ± 1.37°C (SE)), and POM (October-January) (median 27.00°C ± 0.81°C (SE)). The highest water temperature (31.30 °C) was observed on 14 May 2019 at the M3, and the lowest (21.40°C) observed in October 2020 at the M1 (Fig. 4b). A significant variation in the water temperature was observed between PRM and MON

(P=0.02) and PRM and POM (P=0.04) but no significant variation was observed between MON and POM seasons. Spatial variation of the pH (median 7.88 ± 0.13 (SE)) of the estuary was not significant; however, the temporal variation of the pH was significant (K-W chi-square =19.182, DF =2, P<0.001). The pH level of the PRM (median 7.71 ± 0.24 (SE)), MON (median 8.53 ± 0.03 (SE)), and POM (median 7.89 ± 0.14 (SE)) showed significant variation specially between MON and PRM (P<0.001) and between MON and POM (P=0.004). The highest pH (8.64) was observed in October 2019 at the M1 and the lowest (6.00) was observed in June 2020 at the M1 (Fig. 4c). There was significant spatial variation of the *Z. bedoti* population abundance (K-W chi-square = 7.11, DF =2, P = 0.03), specially between the M1 and the M3 (P = 0.03) but not between the M1 and the M2. *Zonosagitta bedoti* population abundance did not vary significantly (K-W chi-square = 4.9, DF = 2, P = 0.17) among PRM, MON and POM seasons; however, the maximum number of individuals (median abundance 1460 ind.m⁻³) was recorded in PRM and the minimum (median abundance 963 ind.m⁻³) observed in POM (Fig.4d). *Zonosagitta bedoti* abundance did not show any significant association with the seasonal changes in the salinity, water temperature and pH of the MR (Table 1).

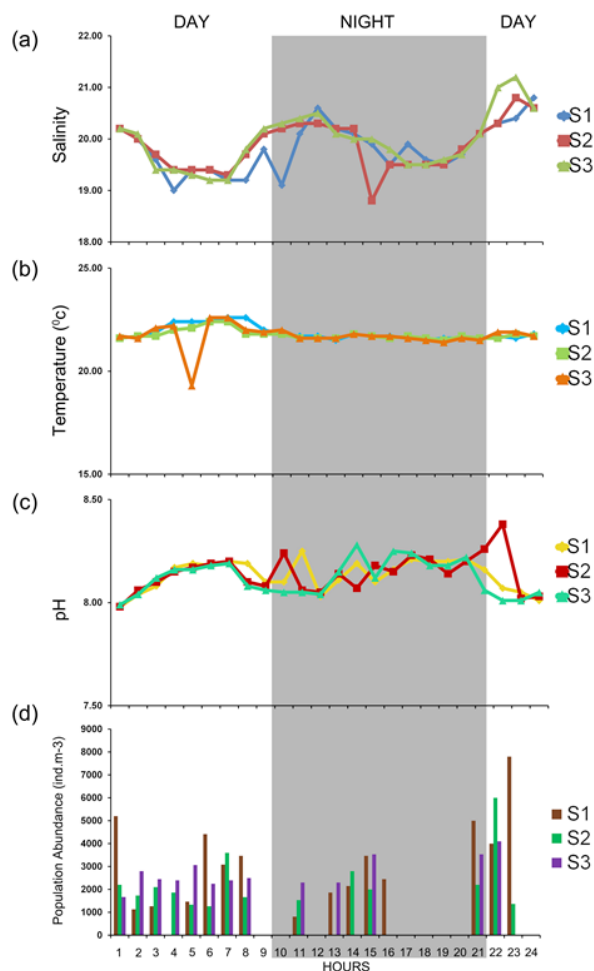


Fig. 3. 24-hour variability of salinity, water temperature (°C), pH and *Zonosagitta bedoti* abundance (ind.m⁻³) in the Saptamukhi river stretch of the Ganges estuary.

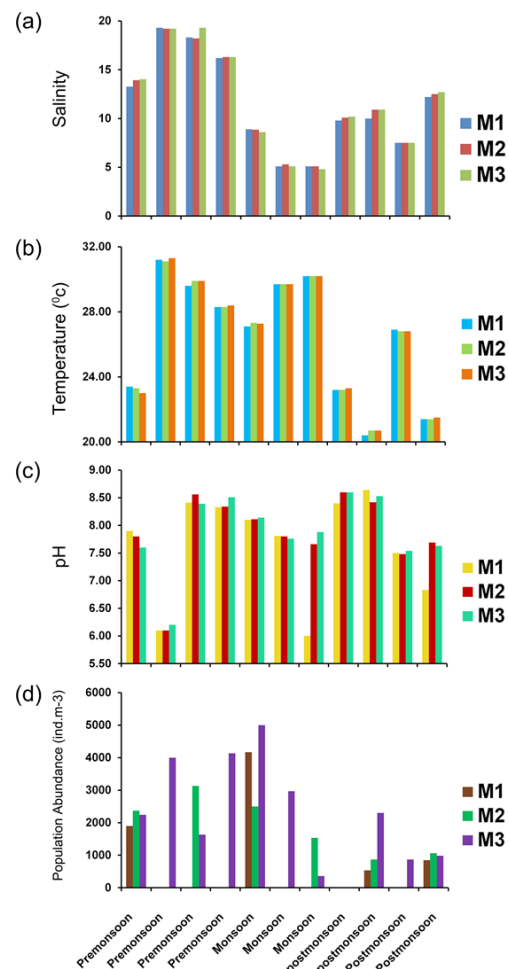


Fig. 4. Seasonal variability of the salinity, water temperature (°C), pH and the *Zonosagitta bedoti* population abundance (ind.m⁻³) in the Muriganga river stretch of the Ganges estuary.

4. Discussion

4.1. Abiotic variability of the micro-habitats

The variability of the abiotic environment at the sampling sites on the MR and the SR was not significant, possibly because the sites were not far from each other. 12-hours and seasonal variations of salinity observed in the MR were similar to those of the previous studies on that stretch of the GE (Sarkar *et al.*, 1986; Mukhopadhyay *et al.*, 2006; Choudhury *et al.*, 2015; Paul *et al.*, 2019). Results suggested that in the MR salinity peaked during the PRM, which closely followed the pattern of the salinity gradient of the Amazon estuaries of Brazil, where salinity shows an inverse relationship with the seasonal rainfall (Costa *et al.*, 2008; Magalhães *et al.*, 2009). Nandy *et al.* (2018) worked on the intra-monsoonal variation of the water quality of the eastern channel of the SR and suggested that the SR is a marine-dominated estuary, which is also evident from the results of the 24-hour study. Seasonal variation of the water temperature of the MR reconfirmed the previous works conducted in the Indian Sundarbans (Bhattacharya *et al.*, 2015; Choudhury *et al.*, 2015; Nandi *et al.*, 2018; Nandy and Mandal, 2020). The seasonal variability of the water temperature of the Indian Sundarbans is not that significant (Bhattacharya *et al.*, 2015), nor is the hourly variability of the water temperature of the MR (Paul *et al.*, 2019). Results suggested that the day-night variation of water temperature is significant in the MR and the SR stretches of the GE. The pH levels of the MR recorded during the 12-hours and the seasonal studies suggested that the MR is slightly alkaline in nature. Those results are consistent with the previous studies conducted on the MR (Mukhopadhyay *et al.*, 2006; Choudhury *et al.*, 2015; Paul *et al.*, 2019). The pH level of the SR section of the GE was also alkaline which is not unusual for the marine-dominated estuaries of the Indian Sundarbans, in particular in the PRM and POM seasons (Bhattacharya *et al.*, 2015; Nandy and Mandal, 2020). Overall, the results suggested that the abiotic variability of the GE is micro-habitat specific (Chaudhury *et al.*, 2015; Nandy *et al.*, 2018; Paul *et al.*, 2019; Chakraborty *et al.*, 2022), which in consequence, may affect its holoplankton, including chaetognaths such as *Z. bedoti*.

4.2. Associations of *Z. bedoti* with the abiotic environment

Zonosagitta bedoti is one of the dominant zooplankton species of the GE (Sarkar *et al.*, 1986). It tolerates a wide salinity range of 1.87 to 31.33‰ in the MR, a wide fluctuation of the salinity (13.1 to 37.5) in the Bombay estuary of India and an even greater salinity range in the Bay of Bengal, Arabian Sea and Red Sea (Sarkar *et al.*, 1986; Nair *et al.*, 1992; Karati and Raveendran, 2012; Karati *et al.*, 2019). In a tidal creek of the Sagar Island (i.e., Chemaguri Creek, which is an offshot of the MR), the relationship between *Z. bedoti* abundance and high salinity was reported by Bhunia and Choudhury (1983). The abundance of *Z. bedoti* generally remains high near an estuary mouth (George, 1952; Sarkar *et al.*, 1986; Li *et al.*, 2009). During the seasonal study, *Z. bedoti* abundance was high at the M3, possibly because the M3 was nearer to the estuary mouth than other sampling sites of the MR.

During the 12-hours study, *Z. bedoti* abundance showed a positive correlation with the salinity but that was not observed in the seasonal study. In the MR, the maximum abundance of the *Z. bedoti* was observed in PRM and the lowest abundance in POM. Bhunia and Choudhury (1983) found the population abundance of *Z. bedoti* was the lowest from July to October (i.e., MON to early PRM) in the MR, which contradicts the current results. Bhunia and Choudhury (1983) observed the peak of the population density of *Z. bedoti* from March to June (i.e. PRM) in the MR, which supports the current results. In the Matla river of the Indian Sundarbans, *Z. bedoti* is present throughout the year; however, its abundance was found to be higher in winter and in spring than in summer and monsoon (Nandy and Mandal, 2020). The 24-hours study conducted on the SR showed that the *Z. bedoti* abundance neither significantly vary with sampling sites nor with the time, possibly because the *Z. bedoti* population is well adapted to the daily abiotic variability of the SR.

Results demonstrated that irrespective of the scale of temporal sampling significant association between *Z. bedoti* abundance and water temperature of the GE does not exist. In the 12-hour study, water temperature varied from 23.03 to 26.50°C and the highest abundance of *Z. bedoti* (3330 ind.m⁻³) was observed when the water temperature was 25.60 °C. In the 24-hour study the highest abundance of *Z. bedoti* (7800 ind.m⁻³) was observed at 21.60 °C. The maximum abundance was observed in PRM when the water temperature of the MR remains high. Such a relationship was also observed by Bhunia and Choudhury (1983) working on the tidal creeks of the Sagar Island which is situated near the mouth of the GE. Sarkar *et al.* (1986) suggested a perennial distribution of the *Z. bedoti* population in the MR irrespective of the temperature profile of the estuary, which supports the present results. In the Bombay estuary, *Z. bedoti* was observed at water temperatures of 24 to 33°C in PRM (Nair *et al.*, 1992). Such an observation closely follows the present results. Water temperature preferences vary among different species of chaetognaths that inhabit various ecosystems of the Bay of Bengal (Nair *et al.*, 2015). In the subtropical waters of the Tolo Harbour of the Hong-Kong, species-specific water temperature preference of the chaetognaths was observed (Tse *et al.*, 2007). *Zonosagitta bedoti* may have a preferred range of water temperature; that not necessarily restricts its distribution in the different micro-habitats of the GE. The population abundance of *Z. bedoti* did not show any significant variation with the temporal change of the pH levels of the MR and the SR. The species possibly tolerates considerable variability of pH in the GE. A nocturnal habit of *Z. bedoti* is well documented and that may relate to prey availability such as copepods (Ghirardelli, 1968; Ball and Miller, 2006; Coston-Clements *et al.*, 2009). However, a nocturnal habit was not observed during the 12-hours and 24-hours studies conducted in the MR and the SR, respectively. In the MR and the SR stretches of the GE, *Z. bedoti* individuals were absent from the surface water during the twilight hours. Reasons for such a behaviour shall be explored in a future study.

4.3. Monitoring *Z. bedoti* populations in Indian estuaries

Chaetognaths are an important component of the planktonic food web and are the valuable indicators of different hydrographic conditions of the coastal-marine environment (Karati *et al.*, 2019). *Zonosagitta bedoti* is a widely distributed member of the zooplankton community and it constitutes a significant biomass in the Indian Sundarbans (Bhunia and Choudhury, 1983; Bhattacharya *et al.*, 2015; Nandy and Mandal, 2020; Chakraborty *et al.* 2022). In the Rio de la Plata estuary of South America, the zooplankton *Acartia tonsa* could be monitored at weekly to monthly intervals without compromising the ecological information related to the habitat (Paul and Calliari, 2019). It is, therefore, important to determine the appropriate temporal scale of monitoring of the *Z. bedoti* population for gaining the insights of the ecological stress of Indian estuaries. *Zonosagitta bedoti* population may be well adapted to the abiotic variability of the specific micro-habitats of the GE. Furthermore, associations between specific abiotic variables (e.g. salinity, water temperature and pH) and *Z. bedoti* population abundance may not necessarily depend on the scale of the temporal sampling. Based on those results and a relatively short life span of the *Z. bedoti* individuals, if a monthly ecological monitoring program (considering both abiotic and biotic interactions together) of *Z. bedoti* population is conceived for a few years than

that may provide a better understanding of their resilience and/or vulnerability in the face of ongoing climate change in the Indian estuaries.

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Compliance with Ethical Standards

Zonosagitta bedoti samples were collected in accordance with the ethical standards of the University of Calcutta, India.

Conflict of Interest

The authors declare that they have no conflicts of interests

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