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SALINITY TOLERANCE TESTS ON SELECTED ESTUARINE COPEPOD SPECIES TO UNDERSTAND THE EFFECT OF DILUTION AND THEIR RESPONSE TO CHANGING ENVIRONMENT

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ABSTRACT

A study was performed to understand the effect of dilution, salinity tolerance and survivability of some selected estuarine copepod species predominant in the lower stretches of the Hooghly estuary at different salinity gradients with respect to time. Acartia erythraea Giesbrecht, Paracalanus indicus Wolfenden, Oithona brevicornis Giesbrecht, Microsetella rosea Dana and Microsetella norvegica Boeck were chosen for the experimental trials. Four experimental trials were performed considering the selected species where different salinity gradients were prepared by diluting the control seawater (collected from the sampling site and in which the copepods were acclimatized) with distilled water for the experimental trials. The experimental trials lasted mostly for 3 - 4 days, since the all the selected species died out of stress. The observations were recorded accordingly. During the course of the study, it was observed that Acartia erythraea was able to tolerate a significant amount of dilution and could withstand a salinity range of 3 – 28 psu, which may confirming its euryhaline nature. Paracalanus indicus was observed to tolerate a salinity range from 10 – 25 psu which may correspond to the salinity of the brackishwater environment. Oithona brevicornis also showed an optimum tolerance of salinity ranging from 15-25 psu. Microsetella rosea and Microsetella norvegica was found to tolerate salinity fluctuations from 12 - 28 psu. It was also observed that *as the salinity gradient experienced considerable dilution, the survivability of copepods decreased with time.*

KEY WORDS - Copepods, Dilution, Hooghly estuary, Salinity gradient, Survivability

1. Introduction

Copepods (meaning 'oar-feet') are a group of small holoplanktonic crustaceans, typically dominating the net zooplankton biomass in the contemporary ocean [1] and have ubiquitous distribution in the global aquatic ecosystem. The adult copepods and their developmental stages are considered to be the most abundant metazoans in the ocean and probably on the planet [2]. Copepods are the most abundant metazoans on the earth [3]. They are a key link between primary producers and the higher trophic level consumers [4] and are important to many higher trophic level consumers as the main natural food resource to larvae and juvenile fish [5] and as prey for ichthyoplankton and other larger pelagic carnivores [3]. They also serve as major grazers of phytoplankton and are components of the microbial loop [3].

In estuaries, tide-mediated diurnal and seasonal salinity variations are important factors affecting the life of aquatic organism who encounter huge salinity stress while moving along with the tidal regime. Stenohaline species that tolerate only a narrow salinity range are limited to the upper and lower ends of estuary, unlike the euryhaline species that tolerate a wide range of salinity gradient and hence found all along the estuarine body [6]. Brackish water habitats are characterized by highly dynamic environmental conditions, primarily salinity fluctuations where dilution of seawater is inevitable [7]. According to the species-minimum concept proposed by [8], the lowest number of taxa occurs at critical salinity levels of 5-8 psu, which is referred to as the "paradox of brackish waters". The organisms living in brackish waters have to tolerate rapid shift in salinity levels, which is why such water bodies are colonized by freshwater and seawater euryhaline species, accompanied by a few typical brackish water taxa [9][10][11].

Variations in salt concentration in both spatial and seasonal scales contribute largely to composition and distribution of zooplankton communities. Salinity is one of the key factors for copepods and affects the spawning, incubation, survival rate, growth and respiration of the copepods and subrogation of the dominant species in nature [12][13][14]. Research has shown that salinity directly affects the physiology of copepods. Experiments have revealed that salinity levels affected the survival and reproduction of copepods such as *Boeckella hamata* with lower survival rates among both male and female adults and a lower egg production rate among female adults [15]. Through experiments with three species of *Acartia*, it was shown that the combination of temperature and salinity affected the survival of copepods, with acclimation helping to increase survival rate [16]. Previous studies have established the relationship between salinity levels and the distribution of the Calanoid copepod, *Acartia tonsa*, by determining salinity tolerance levels [17].

As copepods are sensitive to salinity changes [15][17], they are suitable indicators to assay how salinity changes affect marine health. A study was performed in situ to understand the salinity tolerance of some selected estuarine copepod species. The study may also help in detecting the endemic zones of species traversing upstream and downstream of estuary from their survivability and mortality of copepod species.

2. Material and Method

2.1. Physiography of study area

Sundarban (21°31'E and 22°30'N; 88°10'E and 89°51'E) which means 'beautiful forest' is the largest uninterrupted delta patch in the Ganga-Brahmaputra estuary. The total land area today is 4,143 square kilometres (1,600 sq. miles), including exposed mudflats with a total area of 42 square kilometres (16 sq. miles); the remaining water area of 1,874 square kilometres (724 sq. miles) encompasses rivers, small streams and canals. Rivers in the Sundarbans are confluence zones of salt water and freshwater resulting in the formation of transition zone between the freshwater of the Hooghly and the seawater of the Bay of Bengal. The Sundarban Biosphere reserve occupies an area of about 2585 sq kms of which 1330 sq km is in the relatively undisturbed core area and around 1255 sq km considered as the buffer zone.

Sunderban area observes three distinct seasons viz. premonsoon (March-June), monsoon (July-October) and postmonsoon (November-February). Annual average rainfall ranges from 1900-2100 mm. The average maximum and minimum wind velocities range from 16.7- 20 kmh^{-1} (April-June) and 10.7- 11.8 kmh^{-1} during months of December to February. Sunderban is a tide dominated area where tides are characteristically semi-diurnal with slight diurnal inequality. The flood and ebb currents fluctuate with seasons. The Hooghly River is main offshoot of river Ganga and it carries with itself a huge amount of sediment load that has resulted in the transformation of the deltaic region into irregular marshy coastal habitat. The tidal dominance is experienced upto 250 km i.e. from the mouth to upstream of the river. Being a well mixed estuary, it experiences intense tidal and wave actions with a meso-macrotidal setting (2.5–7 m tidal amplitude) [18][19].

Figure 1 denotes the respective sampling stations chosen for the study. Six sampling stations viz. Kachuberia (21°52.72'N; 88°8.15'E), Chemaguri (21°38'N; 88°08'E), Gangasagar (21°80' N; 88°10'E), Namkhana (21°46'N; 88°14'E), Frasergunj (21°35'55.33"N; 88°14'48.53"E) and Bakkhali (21.5633°N and 88.2594°E) were selected based on their physiochemical parameters mainly focusing on lower stretch of Hoogly estuary along with surrounding anthropogenically disturbed marine and coastal ecosystems beginning with greater freshwater influenced ecosystems near estuary head to gradually brackish water regions at the tide dominated estuary mouth. Kachuberia and Namkhana represented the regions with considerable freshwater influence. Bakkhali and Gangasagar represented the marine dominated regions. Sampling sites at Fraserganj and Chemaguri represented brackishwater environment. The selection of stations was based on the pretext of observing the responses of the biotic communities which are constantly influenced by the ever changing stoichiometry of the ambient media.

2.2. Sample collection

Sampling was performed with the help of country boats at offshore area away from the coast to avoid turbulence and resuspension of the sediments. Air temperature and water temperature were recorded using field thermometer at the sampling site itself. Water samples were collected with the help of Niskin Water Sampler for the estimation of nutrients (mainly nitrate, phosphate and Silicate). The analysis of the nutrients, dissolved oxygen was performed following the standard procedures proposed by [20]. Salinity was recorded on field with the help of Refractometer and then cross checked in the laboratory following argentometric method [21]. pH was recorded on field using portable digital pH meter calibrated at pH buffer 7 using buffer solution.

The mesozooplankton sampling was mainly performed by using zooplankton net of mesh size (65 µM) to avoid the collection of phytoplankton concentrate. The sampling was mainly performed preferably at dawn or dusk as the chances to find greater number of copepod was higher. The net was operated at the starboard side to avoid resuspension and clogging of the net mesh due to sediments. The net was deployed for around 30 mins. After the collection, the mesozooplankton concentrate was transferred to 100 ml Tarsons polyethylene containers. For long term storage of samples, the newly modified combined preservative concentration (2% Formalin + 2.5% Lugol's iodine) [22] was employed so that various ecological measurement can be performed on them without compromising on their natural dimensions, a change induced at times by the preservatives themselves. The primary equipment used for the detection purposes were Olympus brightfield microscope and Nikon phase contrast microscopes. The enumeration was performed with the help of Sedgwick rafter counter chamber. For the identification of Mesozooplankton (copepods) species renowned identification guide of [23][24] were considered.

2.3. Selection of species

Copepods mainly belong to three main group's viz. Calanoida, Cyclopoida and Harpacticoida respectively. Most of the species belonging to these groups can tolerate wide salinity fluctuations. In the course of the study, representative species from each group was selected to understand their extent of salinity tolerance. Moreover, representative subject species might not tolerate the same salinity range as it other counterparts. Different estuarine species have different tolerance limit of salinity. *Acartia erythraea* Giesbrecht, *Oithona brevicornis* Giesbrecht, *Paracalanus indicus* Wolfenden, *Microsetella rosea* Dana and *Microsetella norwegica* Boeck were chosen for the purpose of the study

2.4. Experimental set up

Live copepods were also collected by the similar process as mentioned earlier in the mesozooplankton sampling. The mesozooplankton concentrate obtained after the collection was immediately transferred to the laboratory. Live copepod species were isolated using Leica Stereoscopic Microscope and transferred into filtered seawater. The live samples were kept in dark and allowed to acclimatize prior to the experiment to reduce shock and stress encountered during transportation. After 24 hours the live samples were identified and isolated at species level and then considered for experimental setup. The stock seawater, in which the live copepod species were collected, was used for the preparation of different salinity gradient. Two experimental setups were made. Of them one setup was taken as distilled water which ensured zero salinity and the second experimental set up was taken as the ambient water in which the copepod species were acclimatized. The salinity of ambient water differed both spatially and temporally at the different selected study sites. Several salinity gradients were prepared using sea water (used as stock seawater), and serially diluted using distilled water. The resultant salinity obtained by the dilution considered for the experimental setup was recorded using refractometer. 100 ml beakers were considered for the experiment. Each beaker represented the salinities in the decreasing order. Two beakers were kept as control, distilled water represented zero salinity while the other as the stock seawater.

The main aim was to understand the salinity tolerance of selected copepod species with respect to effect of salinity dilution. Adult copepods of respective species were placed 100 ml beaker of different salinity gradients prepared for the experiment. After 5 minutes of placing the copepods in 100 ml beakers, a first count was conducted on the number of surviving copepods and a second count conducted after 24 hours using Leica brightfield stereoscopic microscope. Species which did not show any kind of movements within the observed period of 10 seconds were considered dead. The experiment trials were performed at the room temperature in which the copepod species were acclimatized. The experimental setup mainly lasted for 96 hours as the most of the copepod species mainly died out of stress. However, while performing the experiments the selected species were kept in well aerated condition to keep the species stress free.

3. Results

During the tenure of the study, different meteorological (Wind Speed, Relative humidity, air temperature water temperature) and physicochemical (pH, salinity, Dissolved Oxygen) parameters were chosen as background reference for the experimental setup. Table 1 shows the monthly variations of the selected background parameters during the course of the study at the different selected study sites. The salinity tolerance of different species was studied and their range of salinity tolerance was observed. The environmental variables were maintained as far as possible to observe the response of the selected estuarine copepods towards changing salinity gradients while performing the experimental trials.

Acartia erythraea and *Paracalanus indicus* were chosen as the representative species of calanoid copepod. *Oithona brevicornis* was chosen as the representative species of cyclopoid copepod, *Microsetella rosea* and *Microsetella norvegica* were chosen as the representative species of Harpacticoid copepod. During the experiment performed, variations in the salinity tolerance were observed among the different copepod species. Experimental setup was maintained at room temperature. Hourly surveillance was done as much as possible to record the mortality of the species.

Figure 2 reflects the first experimental trial that was performed using the selected species. The experiment was performed at room temperature. In this experiment, seven different salinity gradients viz. 25, 20, 17.5, 15, 12.5, 10, 5 and 0 psu were chosen to understand the survivability and salinity tolerance experiment of selected estuarine copepod species with respect to time. The control salinities for the experiment trails were maintained at 25 psu (salinity of the stock seawater) and 0 psu (distilled water). The pH of the experimental set up was maintained at 7.8 which corresponded to the stock water in which the selected copepod species were reared. The salinity of the stock water at the time for the experimental setup was recorded at 25 psu. It was observed that dilution imparted a significant effect on the survivability of the estuarine copepods. As it can be seen from Figure 2, *Acartia erythraea* was able to tolerate wide range of salinity for a minimum of 3 hours and a maximum of 72 hours after which all the copepods died. At zero salinity, all the species introduced in the beaker died of osmotic shock in less than 5 minutes. *Oithona brevicornis* was found to tolerate a salinity range from 15 psu to 25 psu where the survivability was found maximum with respect to time. However, the survivability of the subject species reduced with decrease in salinity. *Paracalanus indicus* was found to tolerate a salinity regime of 10 psu to 25 psu. *Oithona brevicornis* and *Paracalanus indicus* may prefer mostly brackishwater environment. Several other research workers have reported similar kind of observations [25]. The survival and salinity tolerance study using *Microsetella rosea* and *Microsetella norvegica* revealed similar results. Both the species were found to tolerate salinity regime from 12.5 psu to 25 psu. The survival of both the species was found to range from 12 to 72 hours after which all the species died. No species survived at zero salinity.

Figure 3 represents the second experimental trial results observed during the course of the study. The salinity of the stock seawater for the experimental trials was 28 psu. The pH of the stock water used for the study was 8.2. Experiments were carried out at room temperature. From the experiment trials, it was seen all the subject species considered for the experiment survived at control salinity i.e. 28 psu for 96 hours. Considerable level in the dilution of the salinity had a negative impact on the survivability of the estuarine organisms. As observed from Figure 3, The survivability of *Acartia erythraea* reduced as the dilution of the stock seawater increased. *Oithona brevicornis* was observed to tolerate a salinity range of 11.2 to 28 psu.

Although, the survivability of the this cyclopoid species reduced with the increase in dilution of the stock seawater. Maximum survivability was observed within a range of 16.8 psu – 28 psu. *Microsetella rosea* and *Microsetella norvegica* was found to cope up a salinity regime of 11.2 psu to 28 psu. The survivability of *Microsetella rosea* and *Microsetella norvegica* was found to decrease with the increase in dilution of salinity of stock sample water used for the experimental trail. *Paracalanus indicus*, a common calanoid copepod was found to prefer a salinity range range from 16.8 psu – 28 psu which pertains to the brackish to marine water environment. The survivability of the species reduced with gradual dilution in the salinity. No species survived at zero salinity.

Figure 4 represents the results obtained from similar experimental setup performed taking the same subject species. The salinity of stock seawater in which the copepod species were acclimatized was 15 psu. The pH of the experimental trial was maintained at 7.6. Four different salinity gradients (12 psu, 9 psu, 6 psu, 3 psu) were prepared following the protocols of the previous stock seawater that was set as control for the tolerance experiment trial for the selected estuarine species. Zero salinity ensured that there won't be any surviving copepods. Results observed during the following experimental set up stated that *Acartia erythraea* was able to tolerate a salinity fluctuation from 3 psu to 15 psu. The survivability of the A. erythrae reduced due to the increment in the dilution with respect to time. The survivability was found to be maximum at the stock salinity i.e. at 15 psu for 72 hours. *Oithona brevicornis* was able to tolerate a salinity range from 6 psu to 15 psu. However, the species survival time with respect to increase in dilution reduced gradientwise. The survivability of *Microsetella rosea* and *Microsetella norvegica* thrived well between 12 psu to 15 psu but their survival was seemed to reduce due to the dilution factor. *Paracalanus indicus* also showed trend for an optimum tolerance of salinity level from 9 psu to 15 psu. The survivabilty of the species reduced with respect to the dilution.

Figure 5 represents the data for salinity tolerance replicate experiment trial performed using the selected estuarine copepod species. The pH was maintained at 8.3. Salinity gradients were prepared by diluting 20 psu stock seawater as control salinity used for the experiment. During the course of the experiment, it was observed that selected estuarine copepod species survived at the control salinity for 24 hours. Considerable dilution, however, reduced their survival with respect to time. *Acartia erythraea* was able to tolerate significant dilution and was found to survive a salinity regime of 2 – 20 psu. In this experimental trial, the selected copepod species did not survive more than 24 hours which may due to stress the species incurred due to the transportation from the sampling station to the laboratory. The optimum survivability of *Oithona brevicornis*, a cyclopoid copepod was found at 14 psu – 20 psu. *Paracalanus indicus*, another calanoid copepod representative was also found to tolerate a salinity range of 10 psu to 20 psu. The maximum survivability of *Paracalanus indicus* observed during the experimental trial was observed at around 14 psu – 20 psu. Both the species showed a preference to brackishwater salinity. The harpacticoid copepod *Microsetella rosea* and *Microsetella norvegica* was found to tolerate an optimum salinity of 10 – 20 psu. As evident from the figure, *Microsetella rosea* recorded the maximum survivability at salinity range around 10 – 20 psu. Another harpacticoid copepod, *Microsetella norvegica*, recorded maximum survivability between salinity ranges of 10 psu – 20 psu. As dilution of the standing stock water increased the survivability decreased. No species survived at zero salinity which was evident due to the huge osmotic stress encountered.

4. Discussion

In estuaries, the diurnal and seasonal salinity variations are the most crucial factor that affects the coastal faunal life. The fluctuation in the salinity regime limits the distribution of marine organism. Stenohaline species cannot withstand the fluctuations in the salinity range although they may enter the estuarine waters. On the other hand, the euryhaline species can tolerate the salinity fluctuations and are thus considered very important in ecological studies as bioindicators as they can bear the effect of the dynamicity of the estuarine system. The following study was mainly performed with the intention to understand the salinity tolerance of some selected copepod species of three main groups viz. calanoid, harpacticoid and cyclopoid copepods respectively. The study provided a considerable idea regarding the salinity tolerance along with considerable dilution from the stock in which the species were reared. *Acartia erythrae*, *Paracalanus indicus*, *Oithona brevicornis*, *Microsetella rosea* and *Microsetella norvegica* were considered for the study. The salinity tolerance of selected species showed considerable variation in the salinity tolerance and their survivability also showed varied results with respect to salinity tolerance although the chosen species were abundant in brackishwater. The greater survival time at a particular salinity gradient suggests that the particular species might show the nature of the copepod whether it is euryhaline or stenohaline.

Early investigations have shown that *Acartia erythraea*, though mainly a marine and brackishwater tolerate considerable salinity dilution [25]. The present experimental trials performed using *Acartia erythraea* as the subject species conferred to similar results. *Acartia erythraea* was able to tolerate salinity range from 3 – 28 psu. Survival time and salinity gradient showed that *Acartia erythraea* could tolerate a wide range of salinity fluctuation which can correspond to its ubiquitous distribution within the Hooghly estuarine complex. The cosmopolitan distribution of *Acartia erythraea* also reveals that it must have certain osmoregulatory or physiological mechanism that helps it to cope up in the highly dynamic environment [6]. It has been revealed by the works of [26] that calanoid copepods are able to withstand significant level of salinity fluctuation in the mixohaline condition of an estuary, where the conditions are ever changing. Trials performed on *Paracalanus indicus* revealed that it could tolerate a salinity range of 10 – 25 psu which may reveal that *Paracalanus indicus* is best suited and can survive well in brackishwater environment. *Oithona brevicornis,* a cyclopoid copepod was found to tolerate a salinity range from 15 - 25 psu which may reflect that these cyclopoid copepods mostly can thrive in both brackishwater and marine environment. *Microsetella rosea* and *Microsetella norwegica* belonging to harpacticoid group was also found to tolerate a wide range of salinity gradient. Both the representative species were able to tolerate a salinity range from 12 – 28 psu. The results render may render insights that both *Microsetella rosea* and *Microsetella norwegica* may also prefer a brackishwater to predominantly marine environment.

5. Conclusion

Changes in salinity are mostly encountered by the estuarine organisms due to the tidal variations. The mixing of freshwater and seawater results to considerable dilution which greatly influences the osmoregulatory processes of the marine organisms limiting the distribution of aquatic life. Thus, salinity might be considered as a governing factor may limit the distribution of a species. Since temperature and salinity are two important parameter associated with each other, temperature also plays an important role in the distribution of aquatic life. In the present study, five estuarine copepod species were chosen to understand their level of salinity tolerance, since they undergo a significant amount of salinity fluctuation. The present topic undertaken during the course of dissertation was to study the salinity tolerance of some selected copepod species to understand the optimum range of their salinity tolerance. From the study, it was observed that

Acartia erythraea may tolerate a wide range of salinity fluctuation i.e. from 3 psu - 28 psu. The salinity tolerance limit of *Acartia erythraea* was considerably higher than the other subject species chosen for the experiment which means that it can also serve as bioindicators for the changing salinity gradients. *Oithona brevicornis* showed preference towards a salinity range of 15 – 25 psu which generally represents the estuarine salinity range. *Microsetella rosea* and *Microsetella norwegica* showed a salinity tolerance range from 12 – 28 psu. These findings may prove important to understand the influence of salinity as impeding factor limiting the distribution of a particular copepod species and also can serve as important bioindicators for better understanding of the environment. The following study can also pave the path for understand the degree of dilution that may result in death of the copepod species.

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Figure:

Figure 1 - The map is depicting the zones selected as the sites of collection during the field trip. The sites were chosen owing to their varied physico-chemical and biotic nature viz. Bakkhali (Stn.1), Fraserganj (Stn.2) and Gangasagar (Stn. 6) experienced highest marine influence; Namkhana (Stn.3) and Kachuberia (Stn.4) served as regimes with considerable freshwater dominance; Chemaguri (Stn. 5) represented the brackishwater zone.

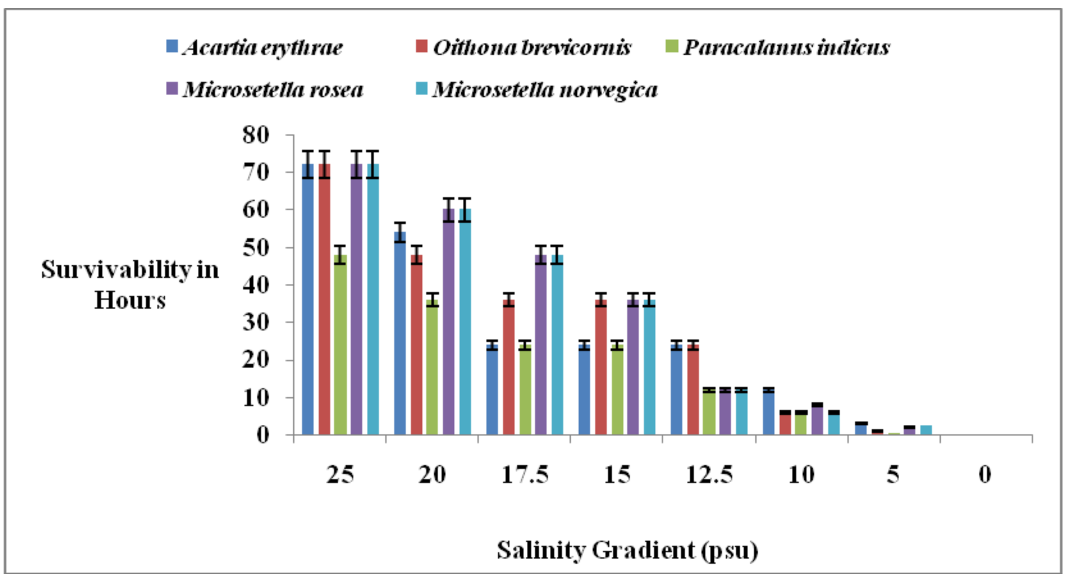

Figure 2 – The following figure represents the response of the five different selected copepod species to the considerable dilution in the different salinity gradients.

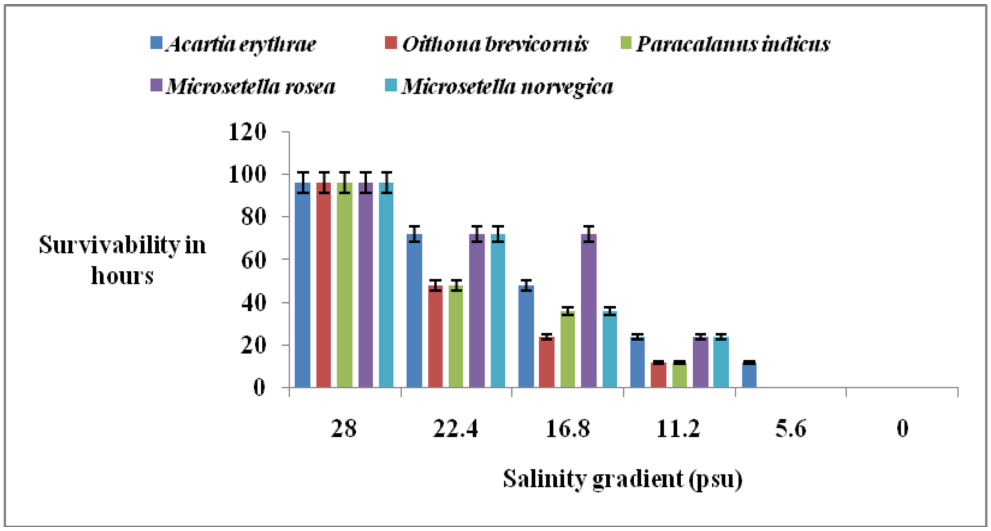

Figure 3 – The following figure shows the different salinity gradients that were prepared to understand the influence of considerable dilution on the selected estuarine copepod species with respect to time.

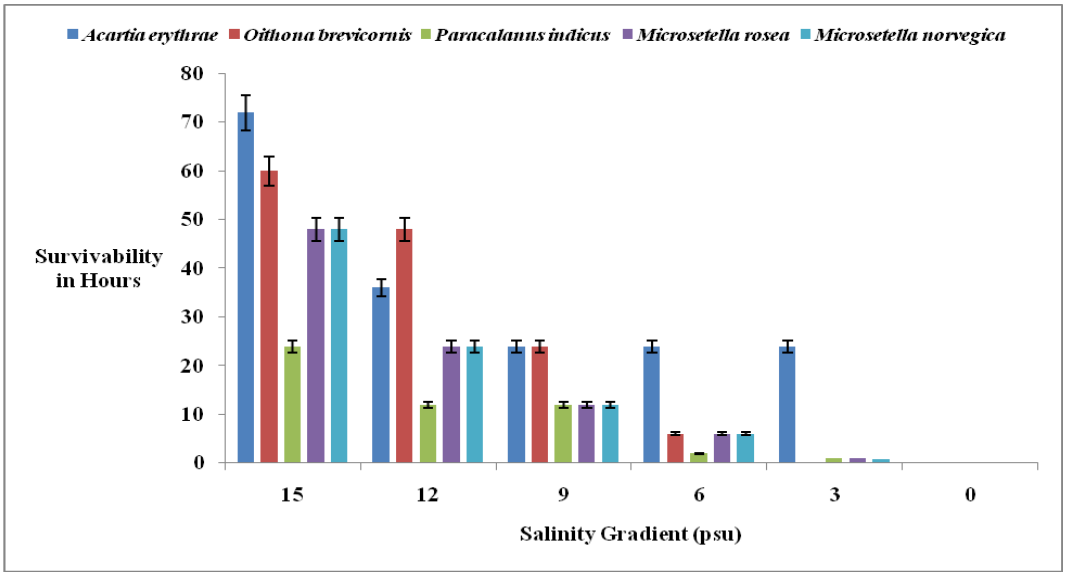

Figure 4 – The following figure shows the different salinity gradients that were prepared to understand the influence of considerable dilution on the selected estuarine copepod species with respect to time.

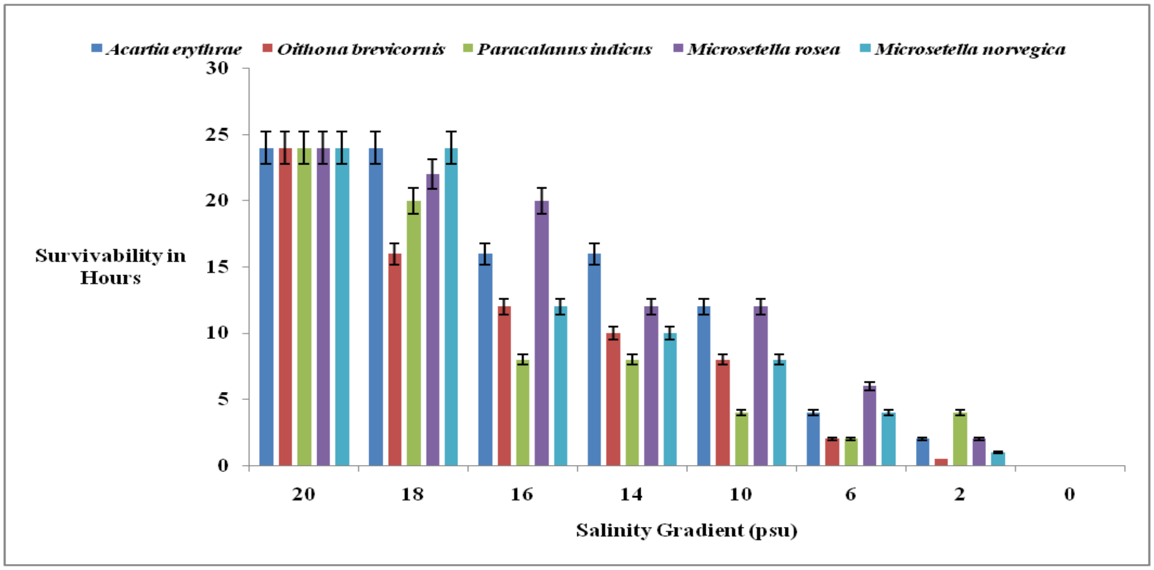

Figure 5 – The following figure shows the different salinity gradients that were prepared to understand the influence of considerable dilution on the selected estuarine copepod species with respect to time.

Table 1: The following table represents the monthly variations in the physical, chemical and the biological parameters of the selected study sites from where the samplings were performed.

Parameters	Month	Stn 1	Stn 2	Stn 3	Stn 4	Stn 5	Stn 6
pH	**Jan'16**	7.8	7.6	8.1	7.5	7.3	7.7
	Feb'16	8.2	8.1	7.7	8.3	8.05	8.2
	Mar'16	7.5	7.8	7.5	7.6	7.6	7.6
	Apr'16	7.5	7.7	7.3	7.5	7.3	7.9
aT (°C)	**Jan'16**	22	22.5	20	22	24.5	25
	Feb'16	25	26	17	26.5	28	28.5
	Mar'16	28	28.5	29	28	29.5	32.1
	Apr'16	26.5	28	31	25.6	27.5	29.5
wT (°C)	**Jan'16**	22.5	22	20	18.5	19.5	20
	Feb'16	22	20	20	20	23	23.5

	Mar'16	25	25.5	26.5	24.5	26	28.5
	Apr'16	27	27.2	28.5	25	26	28.5
Salinity (psu)	**Jan'16**	28	26	16.5	18.5	22.5	25.6
	Feb'16	28	27.9	15	20	24	28.2
	Mar'16	27	27	14	16.5	20	25.5
	Apr'16	27.6	26.2	24	18	20.5	26.5
D.O.	**Jan'16**	6.1	5.1	5.4	5.5	5.5	6.9
	Feb'16	6.3	6.5	6.1	6	5.6	6.5
	Mar'16	5.3	5.5	6.1	6.5	6.3	6.5
	Apr'16	5.2	5.8	5.5	4.5	6.1	6.7

Stn 1 – Bakkhali; Stn 2 – Fraserganj; Stn 3 – Namkhana; Stn 4 – Kachuberia; Stn 5 – Chemaguri; Stn 6 - Gangasagar