

present (Sites 1-5) and five sampling sites where *N. marina* was not present (Sites 6-10) (Fig. 1). 1. Coordinates of these sampling sites are given in Table

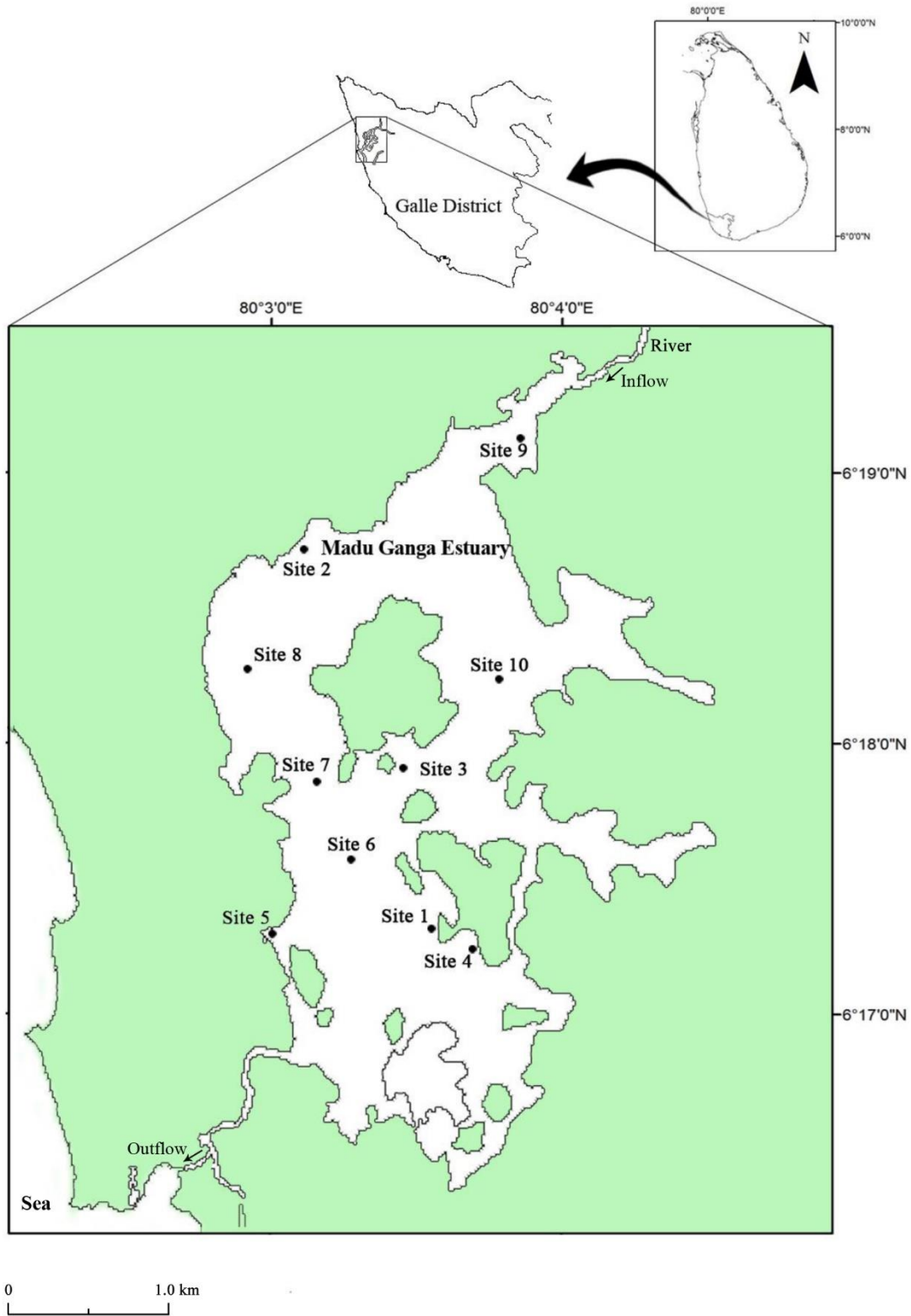


Fig. 1 Map of the Madu Ganga estuary showing the sampling sites

Table 1 Coordinates of the Sampling sites (Sampling sites 1-5 are with *N. marina* and sampling sites 6-10 are without *N. marina*)

Site Number	Latitude	Longitude
1	06° 17.321' N	80° 03.577' E
2	06° 18.732' N	80° 03.116' E
3	06° 17.917' N	80° 03.460' E
4	06° 17.227' N	80° 03.682' E
5	06° 17.290' N	80° 02.997' E
6	06° 17.551' N	80° 03.267' E
7	06° 17.842' N	80° 03.143' E
8	06° 18.285' N	80° 02.838' E
9	06° 19.135' N	80° 03.857' E
10	06° 18.226' N	80° 03.780' E

Depth of the water column at each sampling site was measured using a measuring pole while Secchi depth was measured using a Secchi disc of 20 cm diameter. Surface water temperature, total dissolved solids content (TDS), salinity and pH were measured using a pre-calibrated multimeter (YSI/Model 556 MPS). Dissolved oxygen (DO) content was also measured using a pre-calibrated multimeter (HACH/Model Hq40d multi). From each sampling site, water samples were collected to measure biological oxygen demand (BOD₅), nitrate-N concentration, total dissolved phosphorous concentration (TDP) and total phosphorous concentration (TP) and transported to the laboratory as described by APHA (1998). BOD₅ was measured after incubating at 20°C for five days. Nitrate-N, TDP and TP contents were measured using UV-Visible Spectrophotometer (CECIL/Model: CE 1021) as described by APHA (1998).

The presence or absence of *N. marina* was also recorded throughout the study period at each site. Since data were not normally distributed (Anderson Darling Test; $p > 0.05$), Kruskal-Wallis test followed by Nemenyi test was carried out to compare the physico-chemical parameters temporally and spatially. These statistical analyses were done using MINITAB (Version 14) software package. Considering all physicochemical parameters, cluster analysis was done to check whether the sites with *N. marina* cluster together. Principal Component Analysis (PCA) was carried out to identify the physicochemical parameters that characterize the different sites. PRIMER (Version 5.0) software package was used in the cluster analysis and PCA.

RESULTS

Mean values for the physicochemical parameters for nine months are given in Table 2. Mean depth was the only parameter that was not significantly different in every month. Mean salinity in the estuary was

significantly higher in March and April than in other months ($p < 0.05$). Mean Secchi depth in April was significantly higher than that from May to July and in September. Surface water temperature in April was higher than that of March, May, July, August and October. TDS content in March was higher than that from September to November. Mean pH in April was significantly higher than that in May, July, and August. Mean DO content in April, September and November was significantly higher than that in August. BOD₅ in March and from May to July was significantly higher than that in April and October. Nitrate-N content in March, May and November was significantly higher than that from August to October. TDP content in May and July was significantly higher than that in other months. Mean TP content in July was significantly higher than in October. Mean value of TP in July was significantly higher ($p < 0.05$) than that in October.

Mean values for physicochemical parameters at each site are given in Table 3. There were no significant variation in surface water temperature, TDS, salinity, pH, DO, BOD₅, TDP and TP among sites. Mean depth of sites 2 and 5 was significantly lower than that of sites 6, 7 and 10. Mean Secchi depth at site 2 and site 8 were significantly lower than that of sites 4 and 6. Mean values for nitrate-N in site 1 was significantly higher than that of in site 9. When cluster analysis based on physicochemical parameters is considered (Fig. 2), the 10 sites separate only at a very high similarity level of 94.4%. Eigen values of the PCA are shown in Table 4. 57% of the variances are explained by the principal components 1 and 2. The ordination of the sampling sites based on PC1 and PC2 is shown in Figure 3. Sites 1 and 4 where *N. marina* were present clustered with site 7. Similarly, site 8 where *N. marina* was absent clustered with sites 2 and 3 where *N. marina* was present. Sites 5, 6, 9 and 10 did not cluster with other sites.

Table 2 Monthly Mean \pm SD values for the physicochemical parameters in Madu Ganga estuary from March to November 2014. (T=Surface water temperature; TDS=Total dissolved solids content; DO=Dissolved oxygen content; BOD₅=Biological oxygen demand at day 5; TDP=Total dissolved phosphorus content; TP=Total phosphorus content).

Parameter	March	April	May	June	July	Aug	Sep	Oct	Nov
Depth (m)	2.09 \pm 1.48 ^a	2.26 \pm 1.45 ^a	1.98 \pm 0.62 ^a	1.88 \pm 0.76 ^a	1.70 \pm 0.72 ^a	2.16 \pm 0.66 ^a	1.65 \pm 0.61 ^a	1.59 \pm 0.69 ^a	2.05 \pm 0.65 ^a
Secchi depth (m)	0.96 \pm 0.26 ^{ac}	1.13 \pm 0.20 ^{bc}	0.80 \pm 0.17 ^a	0.83 \pm 0.15 ^a	0.74 \pm 0.19 ^a	0.95 \pm 0.26 ^{ac}	0.80 \pm 0.17 ^a	1.05 \pm 0.33 ^{ac}	1.09 \pm 0.35 ^{ac}
T (°C)	30.88 \pm 0.50 ^b	33.31 \pm 0.96 ^c	28.73 \pm 1.22 ^a	31.13 \pm 0.51 ^{abc}	31.14 \pm 0.43 ^b	29.82 \pm 0.39 ^a	31.30 \pm 0.51 ^{abc}	29.76 \pm 0.44 ^a	30.74 \pm 0.28 ^{abc}
TDS (g/L)	19.21 \pm 0.31 ^e	18.28 \pm .40 ^{cde}	9.29 \pm .66 ^{bcd}	6.68 \pm 1.69 ^{bc}	8.13 \pm 0.55 ^{bcd}	6.12 \pm 0.88 ^{bc}	3.73 \pm 0.69 ^{ab}	4.02 \pm 0.49 ^{ab}	1.91 \pm 0.35 ^a
Salinity (g/L)	19.57 \pm 1.53 ^b	17.51 \pm 0.31 ^b	8.58 \pm 3.09 ^{dc}	5.67 \pm .64 ^{acd}	7.12 \pm 0.53 ^{cd}	6.36 \pm 1.00 ^{cd}	3.11 \pm 0.62 ^a	3.48 \pm 0.27 ^{ad}	1.55 \pm 0.23 ^a
pH	7.73 \pm 0.09 ^{bc}	7.82 \pm 0.11 ^b	7.03 \pm 0.43 ^{ac}	7.45 \pm .33 ^{abc}	5.20 \pm 0.07 ^a	7.00 \pm 0.40 ^{ac}	7.23 \pm 0.11 ^{abc}	8.24 \pm 0.07 ^b	7.19 \pm 0.31 ^{abc}
DO (mg/L)	6.52 \pm 0.4 ^{abc}	7.04 \pm 0.86 ^b	6.34 \pm .07 ^{abc}	6.43 \pm .94 ^{abc}	7.14 \pm 0.49 ^{ab}	6.33 \pm 0.51 ^{ac}	7.22 \pm 0.41 ^b	6.51 \pm 0.10 ^{abc}	7.06 \pm 0.95 ^b
BOD ₅ (mg/L)	1.74 \pm 0.55 ^b	0.93 \pm 0.61 ^a	1.90 \pm 0.44 ^b	1.78 \pm 0.64 ^b	1.62 \pm 0.44 ^b	1.17 \pm 0.32 ^{ab}	1.40 \pm 0.24 ^{ab}	1.06 \pm 0.28 ^a	1.21 \pm 0.28 ^{ab}
Nitrate-N (mg/L)	0.53 \pm 0.24 ^b	0.30 \pm 0.20 ^{ab}	0.49 \pm 0.13 ^b	0.27 \pm 0.13 ^{ab}	0.16 \pm 0.07 ^{ab}	0.13 \pm 0.06 ^a	0.05 \pm 0.03 ^a	0.05 \pm 0.04 ^a	0.61 \pm 0.26 ^b
TDP (mg/L)	Not tested	0.09 \pm 0.04 ^{ac}	0.15 \pm 0.03 ^b	0.11 \pm 0.05 ^{ac}	0.15 \pm 0.03 ^b	0.09 \pm 0.03 ^{ac}	0.08 \pm 0.03 ^{cd}	0.04 \pm 0.01 ^d	0.10 \pm 0.01 ^{ac}
TP (mg/L)	0.13 \pm 0.02 ^{ab}	0.11 \pm 0.02 ^{ab}	0.20 \pm 0.04 ^{ab}	0.16 \pm 0.08 ^{ab}	0.24 \pm 0.06 ^b	0.20 \pm 0.07 ^{ab}	0.14 \pm 0.04 ^{ab}	0.06 \pm 0.01 ^a	0.23 \pm 0.14 ^{ab}

In each row, values denoted by different superscript values are significantly different from each other ($p < 0.05$).

Table 3 Mean \pm SD values for the physicochemical parameters at the 10 sampling sites of Madu Ganga estuary during the study period of March - November 2014. (T=Surface water temperature; TDS=Total dissolved solids content; DO=Dissolved oxygen content; BOD₅=Biological oxygen demand at day 5; TDP=Total dissolved phosphorus content; TP=Total phosphorus content). In each row, values denoted by different superscript values are significantly different from each other ($p < 0.05$).

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
Depth (m)	1.52 \pm 0.45 ^{ab}	1.10 \pm 0.31 ^a	1.56 \pm 0.30 ^{ab}	1.78 \pm 0.29 ^{ab}	1.07 \pm 0.31 ^a	2.66 \pm 0.32 ^b	3.26 \pm 1.39 ^b	1.66 \pm .65 ^{ab}	1.55 \pm 0.26 ^{ab}	2.80 \pm 0.23 ^b
Secchi depth (m)	1.04 \pm 0.23 ^{abc}	0.69 \pm 0.20 ^a	0.92 \pm 0.10 ^{abc}	1.01 \pm 0.28 ^{bc}	0.75 \pm 0.22 ^{ab}	1.13 \pm 0.19 ^c	1.00 \pm 0.17 ^{abc}	0.77 \pm 0.21 ^a	0.81 \pm 0.32 ^{abc}	1.06 \pm 0.24 ^{abc}
T (°C)	31.04 \pm 0.91 ^a	30.83 \pm 2.23 ^a	30.67 \pm 1.19 ^a	30.83 \pm 0.69 ^a	30.54 \pm 1.91 ^a	31.25 \pm 1.22 ^a	30.49 \pm 1.24 ^a	30.70 \pm 1.46 ^a	31.25 \pm 1.57 ^a	30.71 \pm 1.18 ^a
TDS (g/L)	10.23 \pm 5.96 ^a	8.72 \pm 6.25 ^a	9.50 \pm 5.85 ^a	10.39 \pm 6.00 ^a	8.12 \pm 4.65 ^a	8.46 \pm 6.40 ^a	9.79 \pm 5.88 ^a	9.52 \pm 5.70 ^a	7.19 \pm 6.93 ^a	6.66 \pm 5.31 ^a
Salinity (g/L)	9.85 \pm 6.28 ^a	8.13 \pm 5.80 ^a	8.84 \pm 5.53 ^a	9.57 \pm 5.80 ^a	7.37 \pm 4.55 ^a	8.21 \pm 7.02 ^a	9.21 \pm 6.14 ^a	9.16 \pm 6.15 ^a	6.55 \pm 6.59 ^a	6.01 \pm 5.10 ^a
pH	7.22 \pm 0.96 ^a	7.14 \pm 1.15 ^a	7.20 \pm 0.89 ^a	7.13 \pm 0.96 ^a	7.07 \pm 0.93 ^a	7.31 \pm 0.92 ^a	7.24 \pm 0.86 ^a	7.05 \pm 0.99 ^a	7.28 \pm 1.08 ^a	7.42 \pm 1.13 ^a
DO (mg/L)	7.27 \pm 0.91 ^{ab}	5.86 \pm 0.03 ^{ab}	6.54 \pm 0.58 ^{ab}	6.82 \pm 0.50 ^{ab}	6.08 \pm 0.69 ^a	7.15 \pm 0.35 ^{ab}	7.14 \pm 0.29 ^{ab}	7.28 \pm 0.82 ^{ab}	6.50 \pm 1.55 ^{ab}	7.28 \pm 0.26 ^b
BOD ₅ (mg/L)	1.31 \pm 0.36 ^a	1.32 \pm 0.62 ^a	1.44 \pm 0.42 ^a	1.35 \pm 0.65 ^a	1.53 \pm 0.69 ^a	1.16 \pm 0.38 ^a	1.47 \pm 0.58 ^a	1.53 \pm 0.61 ^a	1.56 \pm 0.73 ^a	1.37 \pm 0.49 ^a
Nitrate-N (mg/L)	0.40 \pm 0.29 ^b	0.14 \pm 0.10 ^{ab}	0.27 \pm 0.15 ^{ab}	0.26 \pm 0.19 ^{ab}	0.11 \pm 0.08 ^{ab}	0.26 \pm 0.22 ^{ab}	0.31 \pm 0.26 ^{ab}	0.10 \pm 0.07 ^{ab}	0.07 \pm 0.04 ^a	0.10 \pm 0.0 ^{ab}
TDP (mg/L)	0.09 \pm 0.05 ^a	0.09 \pm 0.04 ^a	0.08 \pm 0.05 ^a	0.09 \pm 0.05 ^a	0.09 \pm 0.05 ^a	0.10 \pm 0.07 ^a	0.11 \pm 0.06 ^a	0.09 \pm 0.03 ^a	0.09 \pm 0.04 ^a	0.08 \pm 0.04 ^a
TP (mg/L)	0.14 \pm 0.09 ^a	0.20 \pm 0.13 ^a	0.14 \pm 0.05 ^a	0.21 \pm 0.14 ^a	0.18 \pm 0.09 ^a	0.21 \pm 0.14 ^a	0.17 \pm 0.08 ^a	0.15 \pm 0.06 ^a	0.14 \pm 0.04 ^a	0.19 \pm 0.13 ^a

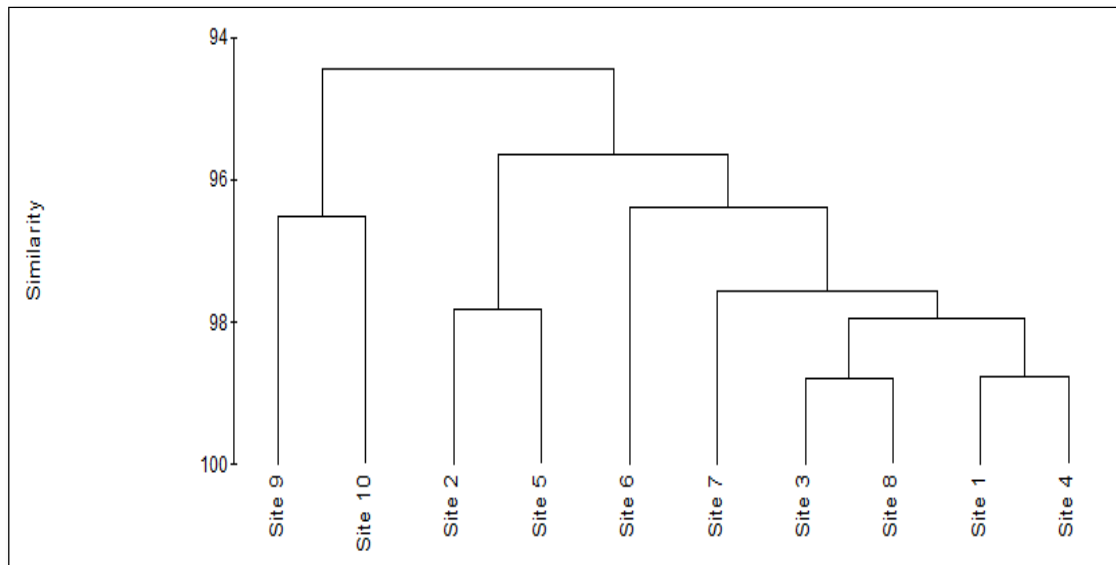


Fig. 2 Cluster Analysis of the sampling sites in Madu Ganga estuary. All the sites were separated out at 94.5% level of similarity

Table 4 Eigenvalues and Eigenvectors obtained from the PCA

	PC1	PC2	PC3	PC4
Eigen value	3.57	2.73	1.76	1.20
% variation	32.4	24.8	16.0	10.9
Cumulative % variation	32.4	57.3	73.2	84.1
Eigenvectors				
Depth	0.450	0.091	-0.229	0.275
Secchi depth	0.471	0.183	0.008	-0.144
TDS	-0.135	0.567	-0.066	-0.075
Salinity	-0.108	0.582	-0.052	-0.115
pH	0.447	-0.215	0.082	-0.126
Temperature	0.165	-0.047	0.422	-0.481
BOD ₅	-0.273	-0.229	-0.543	0.034
Dissolved Oxygen Content	0.374	0.219	-0.324	-0.246
Nitrate concentration	-0.235	0.274	0.417	0.043
Total Dissolved Phosphorous	0.139	0.271	-0.147	0.428
Total Phosphorous	0.174	-0.008	0.400	0.625

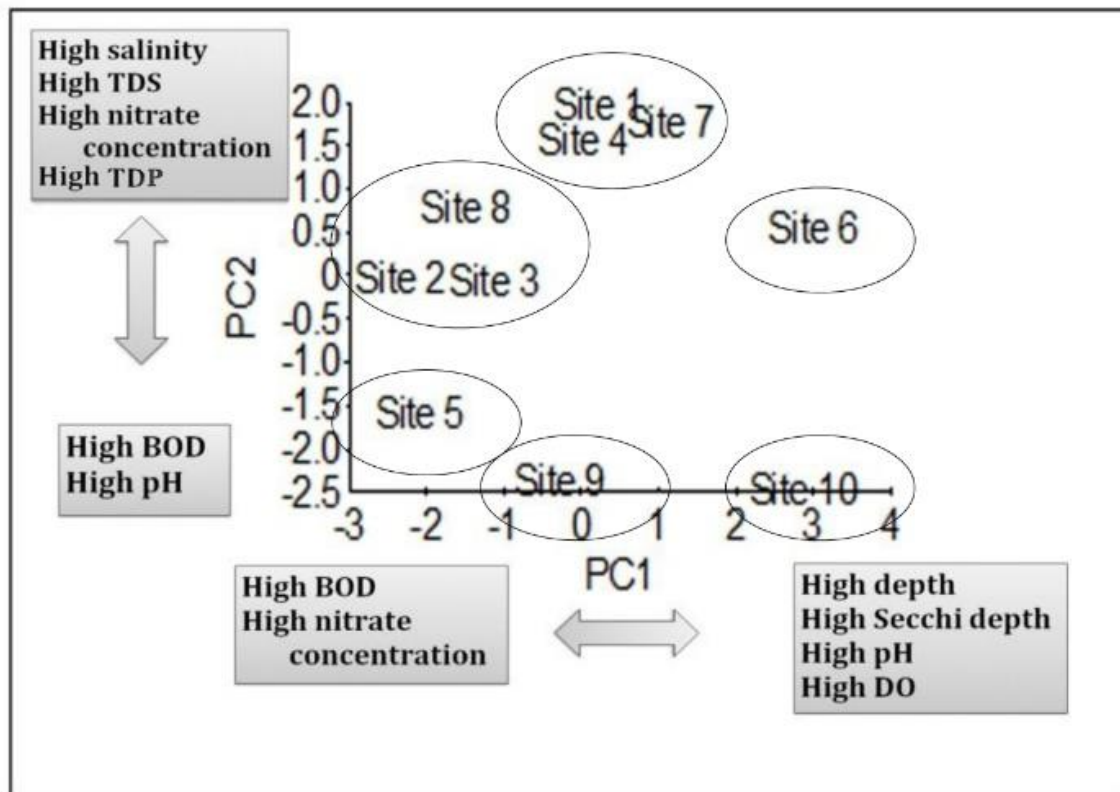


Fig 3. PCA plot showing the clusters

DISCUSSION

During the present study, it was noted that the physicochemical parameters vary within a wide range both spatially and temporally. Similar observations have been made by Amarathunga et al. (2010) in studies carried out in 2006-2007 in this wetland. However, present study indicates that the mean values of most of the physicochemical parameters, namely surface water temperature, TDS, Salinity, pH, BOD₅, TDP and TP were not significantly different among the 10 sites studied ($p > 0.05$). This may be due to high variation of the values observed at each site as evident from the high standard deviation (Table 3).

Non-significant difference in the mean values of most of the physicochemical parameters in different sites indicate that the 10 sampling sites have more or less similar environmental conditions. This was shown by the cluster analysis too as these sites were separated only at a similarity level of 94% (Figure 2). Amarathunga et al. (2010) reported that mean nitrate-N content of the estuary to be 0.26 mgL^{-1} . During the present study, higher values than this were reported in 3 sites namely sites 1, 3 and 6. From March to June, the mean nitrate-N content was higher than that reported by Amarathunga et al. (2010). During the present study, high mean salinity of $17.5\text{-}19.6 \text{ g L}^{-1}$ was recorded in March and April while Amarathunga

et al. (2010) reported the highest salinity of 28.9 g L^{-1} in July. This may be due to temporal variation of the local rainfall in different years.

During the present study *N. marina* was observed in sites 1-5 only. However, none of the physicochemical parameters in those sites were not significantly different from those of other 5 sites, i.e., sites 6-10 where *N. marina* was absent. Therefore, none of the physicochemical parameters studied cannot be considered as individually responsible for the presence or absence of *N. marina* in this estuary. This is also evident from the temporal distribution of *N. marina*. Although *N. marina* was present only from March to May and from September to November, there were no significant differences between the mean values for physicochemical parameters in these months and those of other months.

Mazej and Germ (2008) reported that in Lake Velenjsko Jezero in Slovenia, warm water and unstable sediments were responsible for the successful growth of *N. marina*. However, in Madu Ganga estuary, in all sampling sites, mean surface water temperature was above 30°C . Nevertheless, in some sites, *N. marina* was absent. Hence, it appears that in tropical waters, water temperature does not determine the colonization of *N. marina*. Similarly the area near the freshwater inlet was less prone to the invasion of *N. marina* probably due to high rate of

water flow. In this area sediments were not stable. In September, *N. marina* reappeared first in the areas where the sediments were relatively stable. Hence the observations made during the present study indicate that sediment instability does not promote invasion of *N. marina*.

Although any single physicochemical parameter was not individually responsible for the presence or absence of *N. marina* in any particular area of the estuary, PCA showed that combination of some physicochemical parameters affects the invasion of *N. marina* in this wetland. According to the PCA, some sites such as sites 7 and 8 were identified as the areas that are highly prone to the invasion as they were clustered with the areas with *N. marina*. Such areas were characterized by high salinity, high TDS and high nitrate concentrations. As per PCA, the sites with the least probability to be subjected to the invasion were characterized by high depth (2.80m), high Secchi depth (1.06 m), high pH (7.42) and high DO (7.28 mg/L) that represent areas of relatively low eutrophication (Figure 2). Hence it appeared that the distribution of *N. marina* in Madu Ganga estuary is determined not by a single physicochemical factor but by combination of several factors.

Selig et al. (2007) have shown that in the inner coastal waters of Mecklenburg-Vorpommern in Germany, *N. marina* grows well in the shallow areas of oligotrophic and mesotrophic freshwater lakes where TP levels are < 50 µg/g. Mean TP level in all sites irrespective of the presence or absence of *N. marina* in this wetland was ≥ 140 µg/L indicating eutrophic conditions. PCA also showed that TP is not a significant factor in determining the distribution of *N. marina* in this wetland but high salinity, high TDS, high nitrate-N, low depth, low pH and low DO contribute to the presence of *N. marina* (Table 4; Figure 3). High TDS, high nitrate-N as well as low pH and DO are indicative of eutrophication.

Amarathunga and Sureshkumar (2009) have shown that significant amounts of nutrients are leached into this estuary from the surrounding lands. Several crops including cinnamon, coconut, paddy and vegetable are cultivated in the riparian areas of this estuary. It is well known that the amount of fertilizer used in agricultural activities in many parts of Sri Lanka are very much higher than the required level, so that excess fertilizer are leached into water bodies increasing their nitrate-N and TDP levels (Sangakkara and Wijeyaratne 2015).

Amarathunga et al. (2010) reported that *N. marina* was not present in the upper reaches of the estuary where salinity is zero and also near to sea mouth

where salinity is very high. It appears that intermediate levels of salinity recorded in the present study favours the growth of this invasive species. Although sampling was confined to waters with salinity ranging from 2 – 20 gL⁻¹ where *N. marina* was commonly found, areas close to the sea mouth where the salinity could be expected to be highest, was devoid of *N. marina*, indicating high salinity may prevent/inhibit its colonization/growth. Severed connection to sea by a sand bar formed at the seaward end of this wetland during most of the year contributes to low salinity regime in the estuary that favours the growth and propagation of *N. marina*. Hence, the invasion of *N. marina* in this wetland appears to be affected both by natural environmental conditions such as formation of sand bar resulting in low salinity and anthropogenic activities in the riparian areas such as intense agriculture resulting in eutrophication, as observed in Nasser Lake in Egypt (Yacoub 2009).

Hence, proper cultivation practices without using excess amounts of fertilizer, application of fertilizer at the proper time without allowing them to be leached into this wetland with storm water and controlling soil erosion will help to reduce the nitrate-N loading into this estuary, which will contribute to reduce the invasion of this invasive species in this economically and ecologically important wetland.

Acknowledgements

Authors gratefully acknowledge the financial support provided by the University of Kelaniya. Authors also wish to thank Mr. Krishan Rajapakse of the Department of Zoology and Environmental Management, University of Kelaniya for his contribution in preparing the figures.

REFERENCES

- Amarathunga A.A.D., N. Sureshkumar, K.A.W.S. Weerasekara, W.D.N. Wickramaarachchi and S.A.M. Azmy 2010. Study the effect of salinity and nutrients for the growth of *Najas marina* and its impact to aquatic biodiversity in Madu Ganga Ramsar wetland in Sri Lanka. pp. 155-163. In: Proceedings of the 15th International Forestry and Environment Symposium. Department of Forestry and Environmental Science, University of Sri Jayewardenepura, Sri Lanka.
- Amarathunga A.A.D. and N. Sureshkumar 2009. Comparison of physico-chemical characteristics of the feeding tributaries and nutrient load bringing to Madu Ganga estuary. In: Proceedings of the

- 65th Annual Session of Sri Lanka Association for Advancement of Science, Colombo, Sri Lanka. p. 91.
- APHA 1998. Standards Methods for Examination Water and Waste Water. 20th Ed. American Public Health Association, DC, USA. 1325 pp.
- Bambaradeniya C.N.B. 2002. The status and implications of invasive alien species in Sri Lanka. *Zoos Print Journal* 17(11): 930-935.
- Bambaradeniya C.N.B., S.P. Ekanayake, L.C.D.B. Kekulandala, R.H.S.S. Fernando, V.A.P. Samarawickrama and T.G.M. Priyadharshana 2002. An assessment of the status of biodiversity in the Madu Ganga mangrove estuary. *Occasional Papers* 1, IUCN, Sri Lanka. 49 p.
- Donlan C.J., B.R. Tershy, K. Campbell and F. Cruz 2003. Research for requiems: the need for more collaborative action in eradication of invasive species. *Conservation Biology* 17(6): 1850-1851.
- IUCN and CEA (2006) National wetland directory of Sri Lanka. The World Conservation Union, Central Environmental Authority and International Water Management Institute, Colombo, Sri Lanka. 342 p.
- Joshi C., J. de Leeuwa and I.C. van Durena 2004. Remote Sensing and GIS applications for mapping and spatial modelling of invasive species. *Proceedings of International Society for Photogrammetry and Remote Sensing*. Istanbul. 35: 669-678.
- Kumudinie O.M.C. and M.J.S. Wijeyaratne 2005. Feasibility of controlling accidentally introduced invasive species *Chitala ornata* in Sri Lanka. *Verhandlungen des Internationalen Verein Limnologie* 29: 1025-1027.
- Mazej Z. and M. Germ 2008. Competitive advantages of *Najas marina* L. in a process of littoral colonization in the lake Velenjsko jezero (Slovenija). *Acta Biologica Slovenica* 51(1): 13-20.
- Polley H.W., H.B. Johnson and H.S. Mayeux 1997. Leaf physiology, production, water use, and nitrogen dynamics of the grassland invader *Acacia smallii* at elevated CO₂ concentrations. *Tree Physiology* 17: 89-96.
- Sangakkara S.M.A.I. and M.J.S. Wijeyaratne 2015. Community structures of zooplankton and trophic status of some inland reservoirs in the low country intermediate zone of Sri Lanka. *Sri Lanka Journal of Aquatic Sciences* 20(2): 59-74. doi: <http://doi.org/10.4038/sljas.v20i2.7479>
- Selig U., M. Schubert, A. Eggert, T. Steinhardt, S. Sagert and H. Schubert 2007. The influence of sediments on soft bottom vegetation in inner coastal waters of Mecklenburg-Vorpommern (Germany). *Estuarine, Coastal and Shelf Science* 71(1-2): 241-249.
- Yacoub H. (2009) *Najas* spp. growth in relation to environmental factors in Wadi Allaqi (Nasser Lake, Egypt). *Transylvanian Review of Systematical and Ecological Research* 8: 1-40