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Multifactorial Etiology of Infections by Larvae of *Eustrongylides tubifex* (Nematoda:Dioctophymidae) in Silver Whiting of the Central West Coast of India at Goa

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ABSTRACT

The investigations on ecological dynamics of larvae of *Eustrongylides tubifex* in silver whiting, *Sillago sihama* were conducted at the Central West Coast of India at Goa during 2008-2010.The fish were borrowed from commercial vessels and trawlers as well as purchased from market for the parasitological investigations. All the hosts were brought to laboratory, separated sexwise, freshly weighed, their total length and standard length measured. Viscera of the hosts were teased and carefully examined for helminthes parasites. The collected worms were processed for the morphometric analysis to establish indentify of worms. The dynamics of *E. tubifex* larvae was monitored and impact of environmental parameters established by the application of Principal Component Analysis using SYSTAT 11 software. The dominant 1st component (PCI^F) of Principal Component Analysis elucidated critical negative impact (-0.560) of alkalinity of water on mean intensity of nemic populations in female fish, while the temperature optimum was 28°C, besides influence of seasonality depicting highest peak of infection prevalence in winter and the other lower peak during summer period. The appropriateness of Principal Component Analysis could be substantiated by the cumulative percentage of variance explained by *Sillago sihama- E. tubifex* model that could be used as post-hoc measure. Further, the stability of cumulative percentage in this study, which progressed from 28.190 to 30.167%, suggested that the model became more relevant each year. The robustness of the applications within Principal Component Analysis are strengthened in this study by the availability of two year's samples under natural conditions in an aquatic ecosystem. The higher expression variability of certain select physico-chemical characteristics like, alkalinity, hardness, DO and water temperature were marked out by application of Principal Component Analysis. It also highlighted the reliability of correlation matrix as compared to covariance matrix because of ordered variability structure.

Key words: *Eustrongylides tubifex*, *Sillago sihama*, principal component analysis, central west coast of India, multifactorial, hydrobiological

INTRODUCTION

The dynamics of roundworm infrapopulations in fish of marine water bodies world over, are under the interactive influence of a variety of hydrobiological attributes but the intrinsic changes therein are most misunderstood, that have gained significance in view of the recurrent climate change world over (Hudson *et al.*, 2006). The larvae of *Eustrongylides* are known to inhabit various sites within the host fish, most frequently in the encysted form and these worms usually mature in the interior of birds and mammals in the ambient environment. The developmental dynamics of nematodes is under the influence of salinity because of its direct impact on development and survival of the nematodes in the water body (Kirk *et al.*, 2000; Fazio *et al.*, 2008). Poisson distribution is an appropriate tool to assess overdispersion of parasites within host populations (Anderson, 1991), that ultimately assisted in estimation of regulatory role of parasite burden on the survival of fish hosts. Parasite have also been considered responsible to affect a wide variety of morphological traits of the hosts (Moore, 2002) and pattern of consumption of specific dietary items (Lindenfros *et al.*, 2007), which, in turn could easily influence the seasonality of infestation in fish and other vertebrates. Despite the agreement among parasitologists on the nature of serious effect of parasites on the biology of their hosts, an accurate identification of traits that might be critical, could not be made mainly because of our lack of understanding to substantiate precise influence of individual ecological parameters in Asian waters, on specific host-parasite interrelationships. The present investigation is a two year's study to make an attempt to substantiate affinity of environmental characteristics with host and parasite biology, by using most modern biostatistical tools, in terms of dynamics of nematode infections in a marine fish.

MATERIALS AND METHODS

The investigations were conducted for two consecutive years between July, 2008 to June, 2010 on marine fish *Sillago sihama* (N = 653) sampled at Cacra beach at the Central West Coast of India. The collected hosts were brought to laboratory, separated sex wise, freshly weighed and their total and standard length were measured. The larval forms of *E. tubifex* (N = 2536) were recovered in membranous cover attached to the visceral organs in body cavity of the fish. The larvae were also encountered in the intestine as well as embedded in cystic form in the outer wall of stomach. The cysts were then teased with fine dissecting needles to extract the nematode larvae. The larvae were washed in normal saline (0.85%); fixed in hot alcohol and glycerol (95:5 v/v), cleared in lactophenol after (Malhotra and Rautela, 1984) and mounted in glycerine.

The weekly quantitative estimation of dissolved oxygen in water was done by the modified Winkler's method (Strickland and Parsons, 1968) and water temperature recorded at the site of investigation. Water samples were collected simultaneously in Torson's sampling bottles for hydrobiological analyses of other parameters viz. hardness, acidity, alkalinity and chloride by titrimetric methods after American Public Health Association (APHA, 1998), while salinity was estimated using digital Salinometer (ERMA hand Refractometer, Tokyo, Japan).

The correlation of Infection Prevalence (IP) and Mean Intensity (MI) with various biological attributes of the fish as well as hydrobiological parameters were worked out by using SYSTAT-11 software. Simultaneously, various biostatistical parameters such as Linear regression trends, Principal Component Analysis, ANOVA (one way), Multivariate analysis, Student 't' test and Mann-Whitney's test were also worked out by using advanced biostatistical tools. The dispersion of infection in the marine hosts was calculated by Poisson distribution.

RESULTS

Season and sex of fish: The seasonal abundance of larvae of *E. tubifex* in *S. sihama* indicated typical single peak for mean intensity (20.0) with infection prevalence, 90.91% during 2008-2009 and mean intensity 18.5 with nemic prevalence 83.33% during 2009-2010 was reported in winter period. But a second peak was also encountered in summer period (May-June) of 2008-2009 (15.2) and during 2009-2010 (14.5) (Table 1). Out of the total examined hosts, 21.43-90.91% male fish with mean intensity 2.0-20.0 and 27.27-75.0% female fish with mean intensity 2.29-15.20 were encountered during 2008-2009. However, 25.0-83.33% nemic prevalence with mean intensity, 2.6-18.5 occurred in male fish, and infection prevalence 10.0-70.0% with nemic intensity, 4.2-20.0 occurred in female fish during 2009-2010. The distribution of infection exhibited poor agreement with Poisson series ($p<0.20$, Table 2). Therefore, the nematode populations were over dispersed. The results would thus illustrate restricted distribution of parasitic stages in certain select hosts but the infection would not be spread in fishes all over the water body. The influence of marine habitat characteristics, geographical location and individual physiological state of the host fish have, therefore, predominantly influenced the distribution pattern of these nemic larvae as one of the important factors of multifactorial etiology highlighted in these investigations.

Hydrobiological attributes: The varied trends of infectivity by nematodes are the order of interactive influence of physico-chemical parameters in a water body. Therefore, no single parameter of infection, whether nemic prevalence or mean intensity, is under the influence of a

Table 1: Monthwise distribution data of larvae of *Eustrongylides tubifex* in *Sillago sihama* during 2008-2010

	Host examined			Infection prevalence (%)			Mean intensity		
Months	Male fish	Female fish	Total fish	Male fish	Female fish	Total fish	Male fish	Female fish	Total fish
2008									
July	10	11	21	30.00	27.27	28.57	2.00	3.33	2.67
August	8	17	25	37.50	29.41	32.00	5.33	3.60	4.25
September	16	12	28	43.75	75.00	57.14	5.57	4.22	4.81
October	19	20	39	52.63	75.00	64.10	13.0	9.00	10.6
November	17	11	28	58.82	63.64	60.71	4.40	2.29	3.53
December	15	15	30	66.67	53.33	60.00	13.9	9.88	12.11
2009									
January	11	27	38	90.91	59.26	68.42	20.0	12.0	15.08
February	14	9	23	21.43	55.56	34.78	5.67	3.80	4.50
March	10	15	25	30.00	46.67	40.00	9.33	4.57	6.00
April	15	22	37	40.00	36.36	37.84	12.0	4.00	7.43
May	12	13	25	50.00	38.46	44.00	12.0	15.2	13.46
June	17	8	25	52.94	50.00	52.00	9.45	14.0	12.82
July	6	14	20	0.00	0.00	0.00	0.00	0.00	0.00
August	20	10	30	25.00	10.00	20.00	10.0	14.0	10.67
September	9	12	21	33.33	50.00	42.86	6.00	3.33	4.22
October	15	12	27	46.67	41.67	44.44	8.00	9.00	8.42
November	17	13	30	52.94	30.77	43.33	3.89	20.0	8.85
December	13	10	23	76.92	70.00	73.91	18.5	9.86	14.94
2010									
January	12	10	22	83.33	20.00	54.55	7.20	12.5	8.08
February	10	15	25	30.00	33.33	32.00	6.67	4.20	5.13
March	12	10	22	25.00	30.0	27.27	4.00	10.0	7.00
April	17	12	29	35.29	25.00	31.04	3.33	7.33	4.67
May	14	11	25	42.86	36.36	40.00	11.5	14.5	10.2
June	18	17	35	55.56	35.29	45.71	2.60	6.33	4.00

Table 2: Poisson series of larvae of *Eustrongylides tubifex* in *Sillago sihama* during 2008-2010

No. of Worms	Infected fish	Poisson series*
0	363	234.08
1	12	909.07
2	34	1765.24
3	29	2285.15
4	20	2218.65
5	32	1723.27
6	13	1115.15
7	13	615.02
8	15	300.42
9	14	129.63
10	22	50.35
11	8	17.77
12	12	5.75
13	4	1.72
14	7	4.77×10^{-2}
15	16	1.23×10^{-2}
16	6	3.0×10^{-3}
17	1	6.85×10^{-4}
18	6	1.48×10^{-4}
19	3	3.02×10^{-5}
20	4	5.86×10^{-6}
21	5	1.08×10^{-6}
22	1	1.91×10^{-7}
23	1	3.23×10^{-8}
24	1	5.23×10^{-9}
25	3	8.12×10^{-10}
27	1	1.75×10^{-11}
28	1	2.42×10^{-12}
29	2	3.24×10^{-13}
36	2	1.03×10^{-19}
38	1	1.10×10^{-21}
42	1	9.32×10^{-6}

*p<0.20(Significant)

single environmental parameter. In the current study, the infestation by larvae in female fish inclined during 2008-2009, at higher salinity (Fig. 1) and the augmented degree of incline between 34-35.5 mg L^{-1} influenced the overall incline under salinity effect. However, the mean intensity decreased at higher alkalinity (Fig. 2) during 2008-2009 and the effect was particularly prominent due to effect of alkalinity at >350 mg L^{-1}. The nemic prevalence (Fig. 3) and mean intensity (Fig. 4) in male *S. sihama* declined at corresponding increase in hardness of water during 2009-2010, that was predominantly marked at >6000 mg L^{-1}. The mean intensity of worms in female fish increased at enhanced water temperature (Fig. 5) during 2008-2009.

Mann-whitney's test: The findings on nemic infection prevalence and mean intensity in *S. sihama* by non-parametric Mann-Whitney Test confirmed influence of salinity and Dissolved Oxygen during 2008-2010 as depicted in Appendix 1.

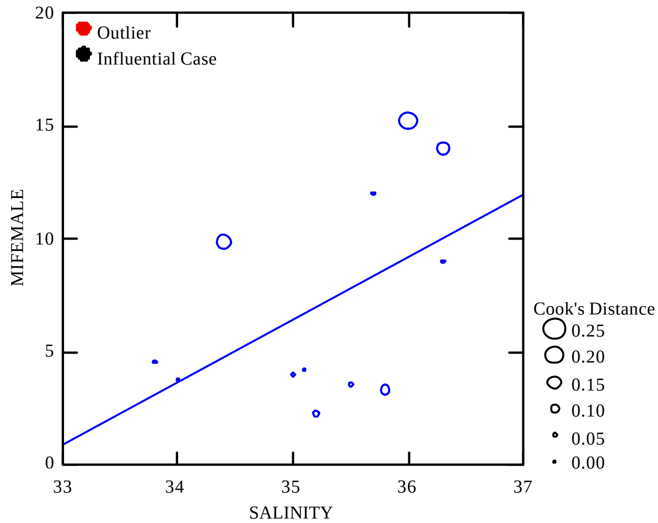

Fig. 1: Correlation of mean intensity by larvae of *Eustrongylides tubifex* in female *Sillago sihama* with salinity (ppt), during 2008-2009

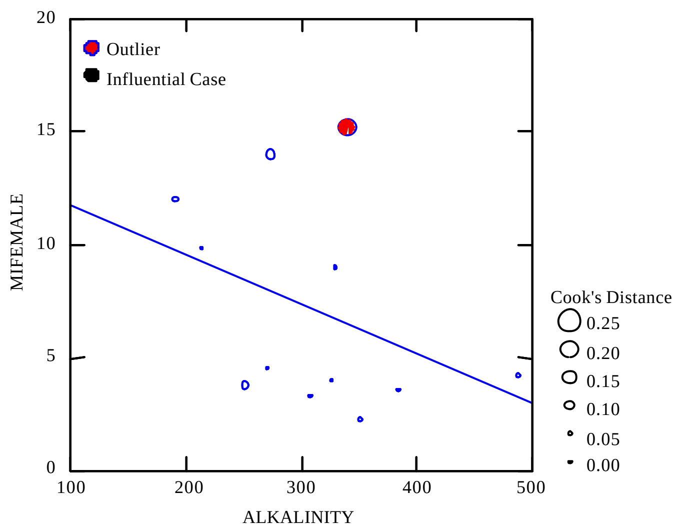

Fig. 2: Correlation of mean intensity by larvae of *Eustrongylides tubifex* in female *Sillago sihama* alkalinity (mg L^{-1}), during 2008-2009

Multivariate analysis: The effect of salinity was predominantly marked on infection prevalence and mean intensity in both the sexes during both years of investigations, except in female fish, where influence of water temperature showed predominant effect over salinity vis-α-vis mean intensity as depicted by the multivariate analysis given below:

2008-2009

IPMALE- r = 0.838

Temp salinity dohardness alkalinity acidity chloride

$$Y=-289.800-4.528X_1+15.956X_2+1.03X_3-0.004X_4-0.168X_5-2.000X_6-0.000X_7$$

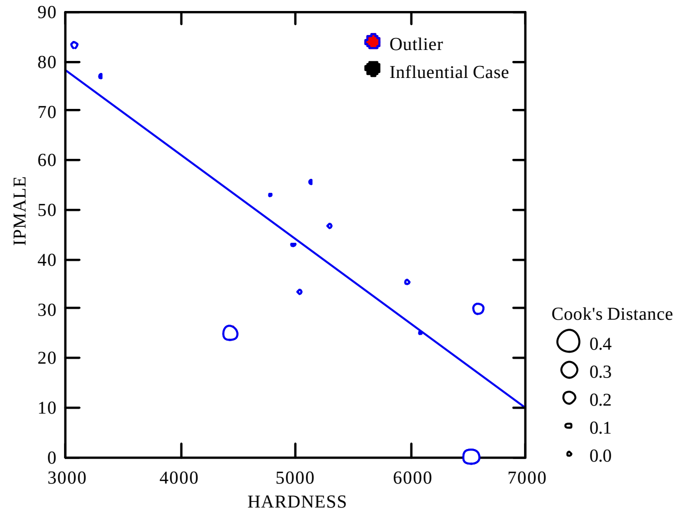

Fig. 3: Correlation of infection prevalence by larvae of *Eustrongylides tubifex* in male *Sillago sihama* hardness (mg L^{-1}), during 2009-2010

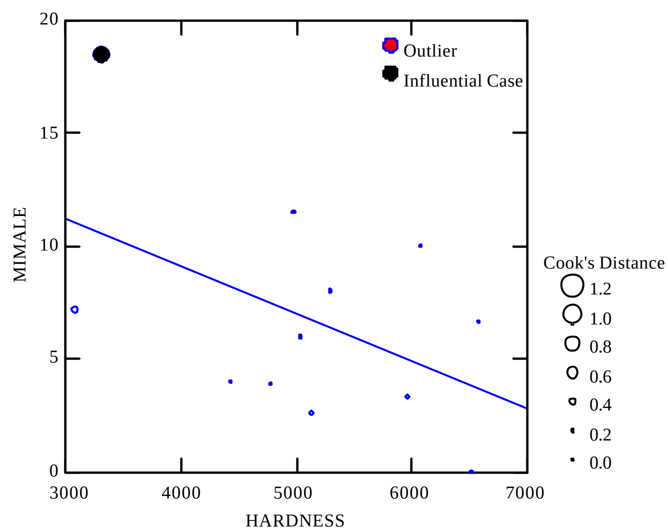

Fig. 4: Correlation of mean intensity by larvae of *Eustrongylides tubifex* in male *Sillago sihama* hardness (mg L^{-1}), during 2009-2010

IPFEMALE- r = 0.809

Temp Salinity DO Hardness Alkalinity Acidity Chloride

$$Y=-69.594-11.304X_1+13.183X_2+5.403X_3+0.003X_4-0.107X_5-4.455X_6+0.001X_7$$

MIMALE- r = 0.801

Temp Salinity DO Hardness Alkalinity Acidity Chloride

$$Y=-14.336-0.878X_1+2.450X_2-1.006X_3-0.001X_4-0.057X_5-0.906X_6-0.000X_7$$

MIFEMALE- r = 0.741

Temp Salinity DO Hardness Alkalinity Acidity Chloride

$$Y=-113.063-0.632X_1+4.300X_2+0.156X_3+0.001X_4-0.039X_5-0.377X_6-0.000X_7$$

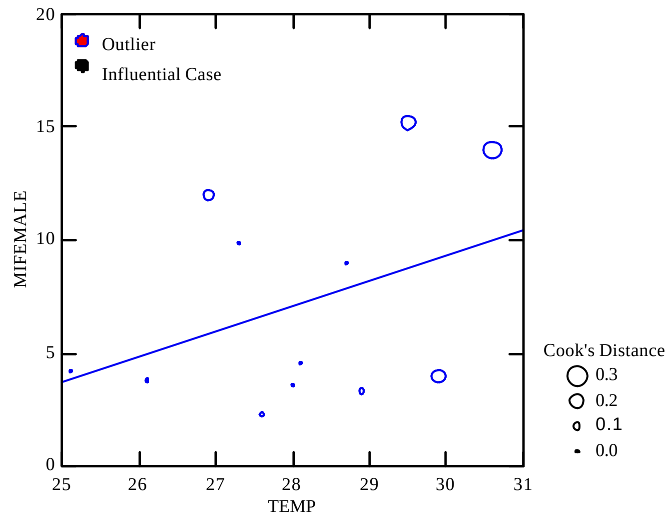

Fig. 5: Correlation of mean intensity by larvae of *Eustrongylides tubifex* in female *Sillago sihama* with water temperature (°C), during 2008-2009

2009-2010

IPMALE- r = 0.858

Temp Salinity DO Hardness Alkalinity Acidity Chloride

$$Y=183.660+4.013X_1-3.081X_2-1.189X_3-0.016X_4-0.113X_5+0.534X_6-0.002X_7$$

IPFEMALE- r = 0.750

Temp Salinity DO Hardness Alkalinity Acidity Chloride

$$Y=389.944+5.698X_1-13.079X_2+8.359X_3-0.011X_4-0.059X_5-1.718X_6-0.000X_7$$

MIMALE- r = 0.663

Temp Salinity DO Hardness Alkalinity Acidity Chloride

$$Y=98.861-0.513X_1-2.668X_2+1.405X_3-0.002X_4-0.014X_5+1.292X_6+0.000X_7$$

MIFEMALE- r = 0.812

Temp Salinity DO Hardness Alkalinity Acidity Chloride

$$Y=-27.821+2.622X_1+0.167X_2-0.352X_3-0.000X_4-0.193X_5+1.762X_6-0.000X_7$$

where, Temp: Water temperature; DO: Dissolved Oxygen

Pearsons's correlation matrix: The influence of water temperature (-0.529) on infection prevalence in female; salinity (0.502) on mean intensity in female; hardness (-0.567) on infection prevalence in male and alkalinity (-0.551) on mean intensity in male were significant during 2008-2009 (Table 3). However, the negative influence of hardness (0.506) on infection prevalence in female fish by larvae of *E. tubifex* was recorded during 2009-2010 (Table 4).

Principal component analysis: The biostatistical assessment of the impact of pollution related parameters, like enhanced salinity was substantiated by the application of Principal Component Analysis and positive coefficient value (0.528) representing mean intensity in female fish (2008-2009)(Table 5).

Table 3: Pearson correlation matrix interrelationship of hydrobiological factors of larvae of *Eustrongylides tubifex* in *Sillago sihama* during 2008-2009

	°C	Salinity	DO	Hardness	Alkalinity	Acidity	Chloride	IPMale	IPFemale	MIMale	MIFemale
Wattemp	1.000										
Salinity	0.502	1.00									
DO	-0.462	-0.30	1.000								
Hardness	0.445	-0.02	-0.359	1.000							
Alkalinity	-0.166	0.201	0.157	-0.124	1.000						
Acidity	0.112	-0.006	0.321	0.164	-0.552	1.000					
Chloride	0.185	0.192	0.086	0.014	-0.099	-0.132	1.000				
Ipmale	-0.093	0.362	-0.037	-0.567	-0.301	0.122	-0.046	1.000			
Ipfemale	-0.529	-0.013	0.268	-0.301	-0.129	-0.021	0.346	0.411	1.000		
Mimale	-0.115	0.140	-0.417	-0.196	-0.551	0.022	-0.071	0.723	0.229	1.000	
Mifemale	-0.386	0.502	-0.375	0.179	-0.380	0.229	0.032	0.556	0.075	0.677	1.000

°C: Water temperature, DO: Dissolved oxygen, IP: Infection prevalence, MI: Mean intensity

Table 4: Pearson correlation matrix interrelationship of hydrobiological factors of larvae of *Eustrongylides tubifex* in *Sillago sihama* during 2009-2010.

	°C	Salinity	DO	Hardness	Alkalinity	Acidity	Chloride	IPMale	IPFemale	MIMale	MIFemale
Wattemp	1.000										
Salinity	0.451	1.00									
DO	0.189	0.17	1.000								
Hardness	0.198	-0.24	0.039	1.000							
Alkalinity	0.596	0.23	-0.23	0.258	1.000						
Acidity	0.258	0.21	-0.23	-0.177	0.597	1.000					
Chloride	0.139	-0.23	0.294	0.299	-0.396	-0.761	1.000				
Ipmale	-0.130	0.21	-0.09	-0.830	-0.128	0.319	-0.422	1.000			
Ipfemale	-0.038	-0.07	0.392	-0.506	-0.342	-0.160	0.151	0.553	1.000		
Mimale	-0.198	-0.16	0.108	-0.482	-0.198	0.224	-0.151	0.528	0.641	1.000	
Mifemale	-0.059	0.14	0.147	-0.435	-0.476	0.145	-0.058	0.468	0.066	0.342	1.000

°C: Water temperature, DO: Dissolved oxygen, IP: Infection prevalence, MI: Mean intensity

The higher expression variability of certain select physico-chemical characteristics like, alkalinity, hardness, DO and water temperature were marked out by application of PCA. It also highlighted the reliability of correlation matrix as compared to covariance matrix because of ordered variability structure, particularly its highest coefficient value (0.886) at enhanced alkalinity (2008-2009). On the other hand, declining effect of hardness (Coefficient value, -0.869) on mean intensity of roundworms in male fish (0.750) during 2009-2010 (Table 6) highlighted the significant principal inorganic ion components as salinity, alkalinity and hardness in parasitological analysis of *E. tubifex* infections in silver whiting in India. The Scree plots in Fig. 6 and factor loadings in Fig. 7 illustrated dominant effect of the 1st component during 2008-2009, and that of the 1st Component during 2009-2010 is depicted by Scree plots (Fig. 8) and factor loadings (Fig. 9).

The inherent variance in the data of distribution of the nematode, *E. tubifex* in Silver whiting was illustrated by the ordered arrangement of Principal Components, that were assigned a specific number label by decreasing order of its contribution to the inherently compiled variance. A Scree plot thus generated exhibited an elbow down the abscissa, that marked point of separation of the most effective Principal Component segregated from other Principal Components, whose effects were representedly masked biostatistically, under the predominant effect of the dominant one.

Table 5: Magnitude of pattern of PC1^P coefficient for sexwise infection by larvae of *Eustrongylides tubifex* in *Sillago sihama* during 2008-2009

Latent Roots (Eigenvalues)

1	2	3	4	5	6	7	8	9	10	11
3.101	2.491	1.691	1.367	0.923	0.717	0.354	0.157	0.093	0.072	0.034

Component loadings

	1	2	3	4
Wattemp	0.453	-0.740	-0.124	0.224
Salinity	0.528	-0.184	-0.509	0.441
DO	-0.518	0.535	0.277	0.289
Hardness	0.028	-0.755	0.331	0.147
Alkalinity	-0.560	-0.037	-0.729	0.141
Acidity	0.197	0.149	0.800	0.308
Chloride	0.115	0.101	0.025	0.830
Ipmale	0.701	0.621	-0.165	-0.060
Ipfemale	0.055	0.743	-0.175	0.292
Mimale	0.838	0.280	0.003	-0.349
Mifemale	0.886	-0.048	0.015	0.059

Variance Explained by Components

1	2	3	4
3.101	2.491	1.691	1.367

Percent of Total Variance Explained

1	2	3	4
28.190	22.643	15.369	12.428

Table 6: Magnitude of pattern of PC1^P coefficient for sexwise infection by larvae of *Eustrongylides tubifex* in *Sillago sihama* during 2009-2010

Latent Roots (Eigenvalues)

1	2	3	4	5	6	7	8	9	10	11
3.318	2.708	1.66	1.159	0.804	0.644	0.306	0.185	0.129	0.085	0.000

Component loadings

	1	2	3	4
Wattemp	-0.280	-0.417	0.750	0.105
Salinity	0.107	-0.458	0.599	-0.432
DO	0.093	0.376	0.702	0.059
Hardness	-0.869	0.103	-0.016	0.115
Alkalinity	-0.388	-0.804	0.138	0.359
Acidity	0.227	-0.868	-0.079	0.102
Chloride	0.319	0.765	0.330	0.021
Ipmale	0.894	-0.236	-0.001	-0.015
Ipfemale	0.665	0.349	0.316	0.518
Mimale	0.752	0.070	-0.032	0.423
Mifemale	0.595	0.069	0.098	-0.598

Variance Explained by Components

1	2	3	4
3.318	2.708	1.661	1.159

Percent of Total Variance Explained

1	2	3	4
30.167	24.621	15.101	10.536

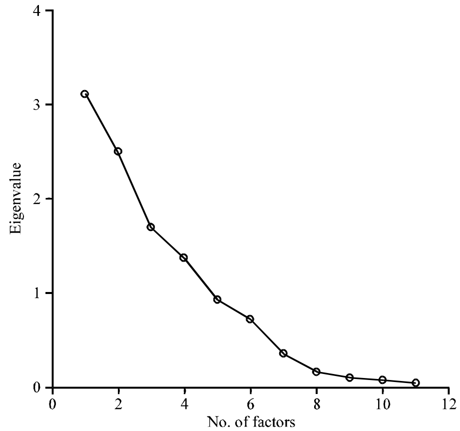

Fig. 6: Scree plot of principal component analysis of larvae of *Eustrongylides tubifex* in *Sillago sihama* during 2008-2009

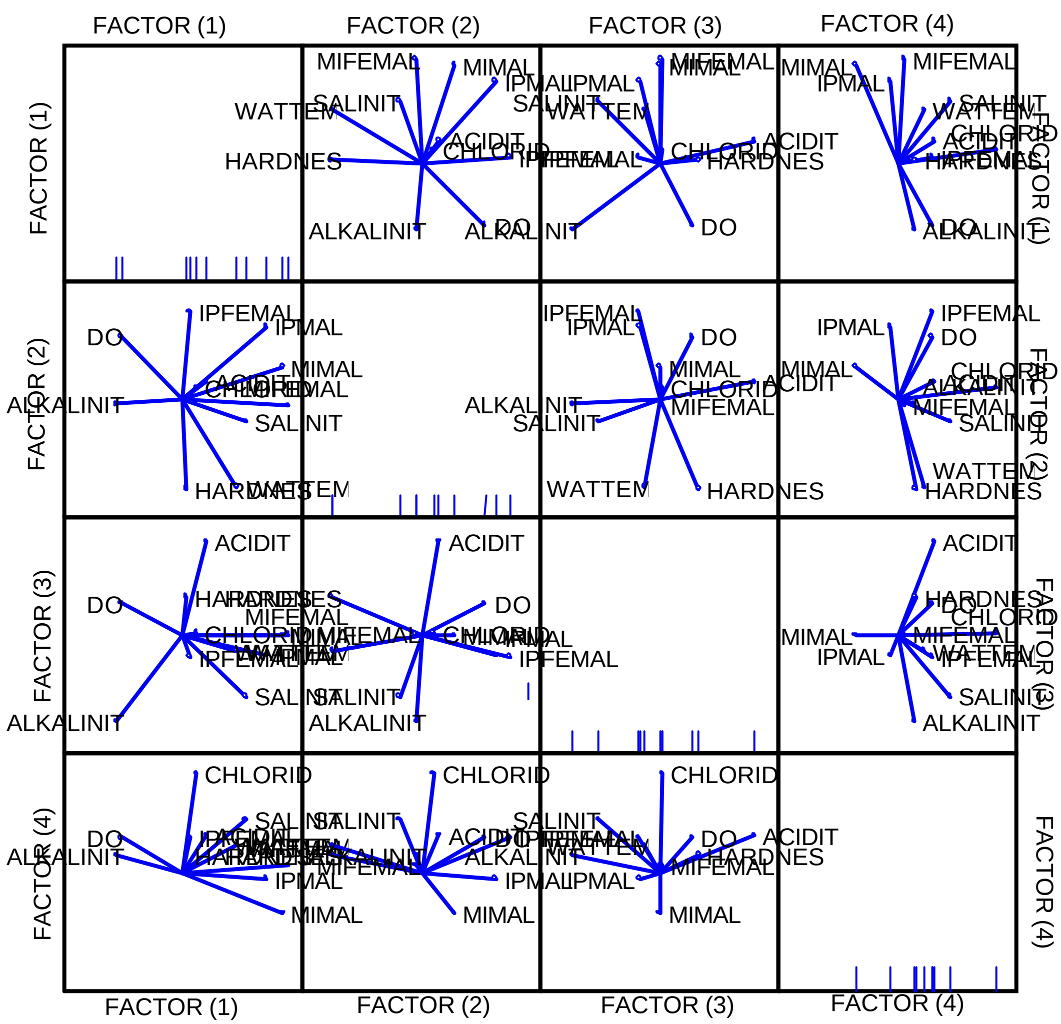

Fig. 7: Factor loadings plot of infection parameters by larvae of *Eustrongylides tubifex* in *Sillago sihama* during 2008-2009

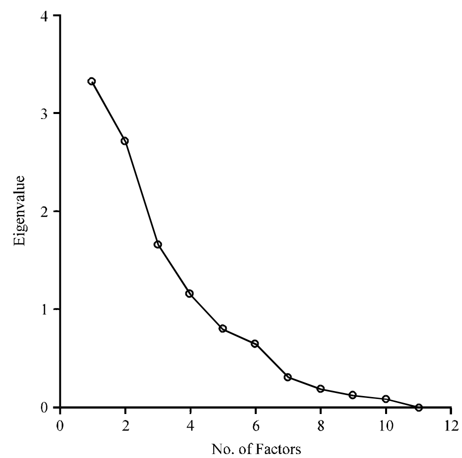

Fig. 8: Scree plot of principal component analysis of larvae of *Eustrongylides tubifex* in *Sillago sihama* during 2009-2010

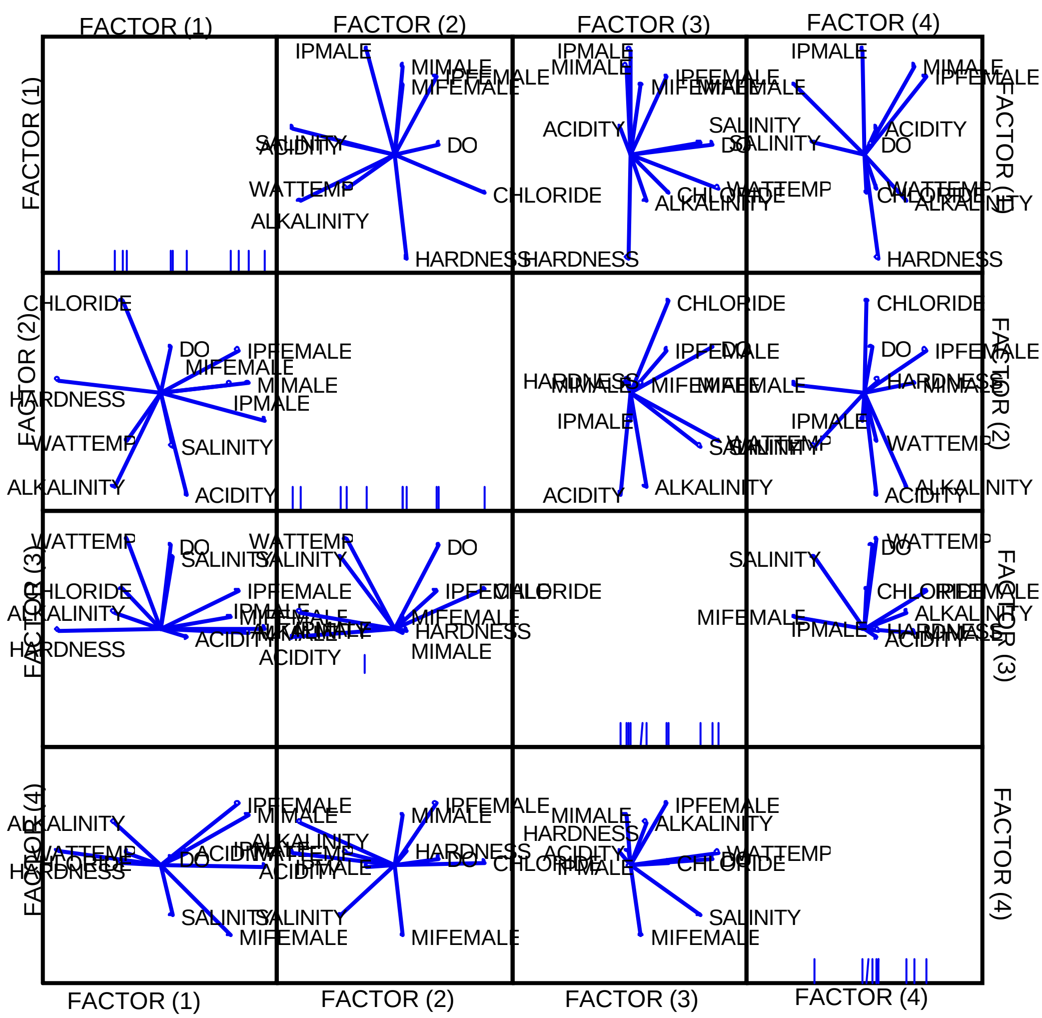

Fig. 9: Factor loadings plot of infection parameters by larvae of *Eustrongylides tubifex* in *Sillago sihama* during 2009-2010

Therefore, the Scree plot projected restricted number of PCS in the predominant condition under natural environment, as against their contribution to mask the effect of other subsidiary ones in effect. In interpretation, the eigenvalues <0.1 are usually not very helpful in statistical terms to derive any conclusions on pattern of distribution of nematodes vis-α-vis factors of the hydrological environment. However, the Principal Components with higher eigenvalues occupied a higher location on the Scree plot and the Factor Loadings plot. The decreasing fractions of total variance explained helped to assort Principal Components displayed in decreasing order on 'X' axis of the Scree Plot. The 'elbow' commenced at PC3 (Started at PC3 and was distinctly noticeable at PC5 onwards till PC11) (Fig. 6 for 2008-2009 and Fig. 8 for 2009-2010), thus outlining noticeable effect of the 1st and 2nd Principal Component. Simultaneously, the non-significant influence of PC3 onwards, till PC11 was marked.

The robustness of the applications within Principal Component Analysis are strengthened in this study by the availability of two year's samples under natural conditions in an aquatic ecosystem. Pedhazur and Schmelkin (1991) postulated that, as a rule of thumb, 50% of the variance should be explained by the first two or three components, to which very closer results were computed in these investigations. In such manner, the appropriateness of Principal Component Analysis could be substantiated by the cumulative percentage of variance explained by this model which could be used as post-hoc measure. Further, the stability of cumulative percentage in this study, which progressed from 28.190 to 30.167%, suggested that the model became more relevant each year. With all 4 indicators of infection prevalence and mean intensity loading significantly, on the first factor during the two years (2008-09: IP male- 0.701, MI male- 0.838, MI female, 0.886; 2009-2010: IP female- 0.665, MI male- 0.752, MI female- 0.595), its strength and general character seem confirmed. In addition, the first factor loadings for alkalinity (-0.560), hardness (-0.869) and salinity (-0.528) also supported the strength of conclusive outcome of the study, that have already been documented in the foregoing text (Fig. 6, 8; Table 5 for 2008-2009 and Fig. 7, 9; Table 6 for 2009-2010).

DISCUSSION

Season and sex of fish: The sustained infection peaks were encountered in late autumn to winter period in female as well as during winter in male (O'Sullivan *et al.*, 1984; Montgomery and Montgomery, 1988). Simultaneously, appreciably higher level of infection throughout the year illustrated that this was due to the continuous availability of annelid intermediate hosts for continuous occurrence of larvae of *E. tubifex* during most part of the year, that further increased during late autumn to winter period. This was in consonance with the studies of Kennedy and Fitch (1990) and Gollock *et al.* (2005). There was single peak of mean intensity in both the sexes of *S. sihama* during winter, as was also concluded by Gollety *et al.* (2005). However, a second peak was also observed in summer (May-June) period (Salvati *et al.*, 2002; Cattadori *et al.*, 2005; Loot *et al.*, 2007), though such a phenomenon was not influenced only due to the season and sex, but also by some other extrinsic factors. A demographic pattern of parasite populations could be concluded in which dynamics of populations interacted with spatial and temporal variations in the environment (Anderson and Gordon, 1982; Nee *et al.*, 1991). The voracious host feeding habits and local habitat characteristics, availability of appropriate intermediate hosts, niche, size of population, spatial features of habitats were suggested as some of the main factors contributing to the variability observed in community composition and richness by earlier workers (Martinez-Aquino *et al.*, 2007).

There was higher infection in female fish than males in 2008-2009, as the report of Pence and Meinzer (1979) also found that the prevalence of *Taenia leonine* was significantly higher in female than males in west Texas. It was due to the positive interaction between endocrine and immune parameters that females harboured lower levels of infection and infestation than male fish during the current investigations. Such factors could contribute to the immune systems of carp being more effective during spawning season (Saha *et al*., 2002). Therefore, female fish were more immunized due to higher level of cortisol (F), a major glucocorticoid, which often increased in the plasma of the spawning fish (Schmidt and Idler, 1962; Wingfield and Grimm, 1977; Maule *et al*., 1996). So, the female fish was more healthy and resistant for the helminthes infection than that of the male fish.

One major finding on Poisson series was that parasite assemblages were not the result of random processes ($p<0.20$), but rather the result of the interplay among geographical location, habitat characteristics and host identity (Kennedy and Hartvigsen, 2000; Nelson and Dick, 2002; Calvete *et al*., 2004). It was demonstrated by Krasnov *et al*. (2008) that the external environmental influence critically determined the extent and variety of invasion by helminth parasites, because of its initial influence on host populations, while the frequent recurrence of parasitic communities depended, in turn, on the effect of host's physiological response to parasitic invasion.

Hydrobiological parameters: The regulatory influence of Dissolved oxygen of marine water body on mean intensity by larvae of *E. tubifex* in male ($Y = 20.74-1.96\ X$; $r = -0.50$, $p<0.40$) and female fish ($Y = 16.42-1.60\ X$; $r = -0.50$, $p<0.50$) was very well marked and showed negative association during 2008-2009. These findings were supported by the conclusions of Zargar *et al*. (2011) on *Diplozoon kashmirensis*. However, the pattern of nemic prevalence in female fish during 2009-2010 was dependent of the effect of Dissolved oxygen (Kuris *et al*., 1980).

The available literature also supported that the frequency of infection was greater due to the temperate regions having served as the most suitable habitat for parasitizes carp (Brock, 1983). But, simultaneously, the establishment of developmental stages resulted in increase of mean intensity in female fish at augmented water temperature ($Y = 24.22+1.12\ X$; $r = 0.50$, $p<0.40$; Fig. 5) during 2008-2009. Kovskii and Khudolei (1989) concluded from their study a direct relation in temperature and parasitic infection, exhibiting positive association between the two. These observations were in agreement with the work reported herein, as the rate of infection increased with increase in temperature. It was also emphasized by Chubb (1980) and Ernst *et al*. (2005) that temperature increased the growth period of parasites and shortened the generation time. Under these conditions, the parasites were in a position to complete their life cycle rapidly. The enhanced temperature could not support the infection prevalence in female fish, because the lymphocyte count in plasma of fish increased at the higher temperature in female fish with simultaneous increase in the cortisol level, that was responsible for the immunity of fish against the nemic infection (Saha *et al*., 2002).

The augmentation in salinity supported the increase in mean intensity in female *S. sihama* (Fig. 1) during 2008-2009. This finding derived support from the contention of Dogiel *et al*. (1961), Snieszko (1974), Chubb (1980), Beer and German (1993), Kennedy and Watt (1994), Marcogliese (2001) and Lafferty and Kuris (2005) that anthropogenic activities were responsible for the eutrophication and thus provided favourable conditions for the emergence of more annelids larvae as intermediate hosts for larvae of *E. tubifex*. Therefore, the voracious feeding of *S. sihama* during that period was apparently responsible for the greater prevalence of infection. No effect of acidity and chloride on the infection prevalence and mean intensity was concluded in the two year's studies.

The multivariate analysis illustrated that salinity masked the effect of other hydrobiological parameters and had significant association with infection prevalence in male ($r = 0.838$) and female ($r = 0.809$) as well as with mean intensity in male ($r = 0.801$) and female ($r = 0.741$) during 2008-2009. However, water temperature co-ordinated with influence of salinity to mask the impact of other hydrobiological factors on the infection prevalence in male ($r = 0.858$) and mean intensity in female ($r = 0.812$).

However, significant masking influence of Dissolved oxygen on infection prevalence in female ($r = 0.750$) and salinity on mean intensity in male *S. sihama* ($r = 0.663$) was noticed on the other physico-chemical factors during 2009-2010.

The principle advantage of application of principle component analysis was to provide an insight to explain variability that was an outcome of a gamut of interactions between host-parasite and hydrological environment.

PCA facilitated to mask singularity and thus exposed multiplex of directions of variability operative within an aquatic ecosystem to support fish-*E. tubifex* interactions at the differential level under the combined influence of a variety of hydrobiological attributes opearating at the same point of time and in several directions to effectuate different dimensions. The higher expression variability of certain select physico-chemical characteristics like, alkalinity, hardness, DO and water temperature were marked out by application of PCA. It also highlighted the reliability of correlation matrix as compared to covariance matrix because of ordered variability structure.

Principal component analysis: The dominant 1st component ($PC1^{p}$) could be deduced from the scree plot of Principal Component Analysis of monthwise response of infection data by *E. tubifex* in *S. sihama* during 2008-2009 (Fig. 6; Table 5) and 2009-2010 (Fig. 8; Table 4.) as well as factor loadings plot during 2008-2009 (Fig. 7) and 2009-2010 (Fig. 9). The influence of alkalinity (-0.560) had a critical impact on the oscillations in monthly and seasonal cycles of nemic mean intensity in female fish (0.886; Table 5). Identical evidence emerged from linear regression analysis between nemic mean intensity in female fish *vis-a-vis* alkalinity ($Y = 13.95-0.02\,X$, $r = 0.50$, $p<0.05$, $F_{1,10} = 1.642$, $S_{0.250}$; Fig. 2). The indirect discriminatory attributes of the hydrological environment like alkalinity, that contributed to a variety of structural inequalities (Wrench, 2007), by their neutral actions, influenced the action of direct discriminatory factors of hormonal intereference by the female sex. These findings were supported by the earlier studies of Engelbrecht (1958) on Eustrongylid infections in fishes of reservoirs of thermal power plants and by Malhotra and Banerjee (1990) on plerocercoid infections in the fishes of Indian Gangetic plains reporting significant decline at increased alkalinity. The analysis of targeted 'mechanism' with an outreach into the step by step operations of an aggregated group of environmental factors, as well as the 'mechanism' of their initiation i.e., 'trigger' has been approached by putting into statistical test, the data generated from naturally occurring interactions within the host-parasite system that is a part of a specific ecosystem, for instance, aquatic ecosystem in the current investigations. The outcome is particularly significant because the data might otherwise seem to be with potentially non-discriminatory, yet the interactive resultant effect could reveal meaningful exclusionary and inclusionary dimensions. The study provided an opportunity to understand that among a voluminous data on environmental sets being available, a select few attributes could eventually be active to influence the pathway of a particular parasite, *E. tubifex,* in this case. In the similar fashion, the association of salinity (0.528) with mean intensity in female fish hosts could be explained by 28.190% total explained variance, as also confirmed by the significance obtained (r = 0.741) with multivariate analysis and linear regression trend (Y = 89.67+2.75 X, r = 0.50, $p<0.50$, $F_{1,10} = 3.377$, $S_{0.100}$ Fig. 1) during 2008-2009. Pearson correlation matrix also exhibited high significance (0.502) with salinity during 2008-2009 (Table 3). In the following year, 2009-2010, Principal Component Analysis of infection variables of mean intensity by nematode larvae in male fish (0.750) under influence of hardness of riverine water (-0.869; Table 6) showed a first component ($PC1^{P}$) accounting for 30.167% (Fig. 7, 8). This was in conformity to the finding of significant linear regression trend between hardness and nemic intensity in male fish (Fig. 4). The declining influence of hardness on the mean nemic intensity (Y = 17.53-0.002 X, r = -0.50, $p<0.025$, $F_{1,10} = 3.023$ $S_{0.250}$; Pearson correlation matrix, -0.482; Fig. 4) and infection prevalence in male fish (Y = 129.32-0.02 X, r = -0.82, $p<0.40$, $F_{1,10} = 22.201$, $S_{0.005}$; Pearson correlation matrix, -0.830; Fig. 3) during 2009-2010 were also noticeable. Therefore, the biostatistical correlations worked out in the current investigation highlighted the relatively significant influence of salinity, alkalinity, hardness and Dissolved oxygen as well as water temperature in the dynamics of infrapopulations of *E. tubifex* infections in marine fish, *S. sihama* at the Central West Coast of India at Goa. Navara (2009) attributed such influence to the resource availability and consumer density differentiation pattern due to the effect of latitudinal variation in climatic factors between temperate and tropical regions. Therefore, the study based on the analysis of environmental data related to a host fish-*E. tubifex* infections provided evidence of the existing constituents of structural inequalities in natural water body, comprising alkalinity, hardness, salinity, dissolved oxygen and water temperature, having triggered larval growth following patterns, that were demonstrated herein, and predominantly thus, these structural inequalities affected the role of direct discriminators of host biology that deduced specific nemic pathways concluded in these investigations.

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APPENDIX

APPENDIX 1

2008-2009

Mann-Whitney One-Way Analysis			Mann-Whitney One-Way Analysis		
of Variance for 12 cases			of Variance for 12 cases		
Dependent variable is IPFEMALE			Dependent variable is IPMALE		
Grouping variable is SALINITY			Grouping variable is DO		
Group	Count	Rank Sum	Group	Count	Rank Sum
33.8	1	5.000	4.5	2	17.000
34	1	8.000	4.8	1	7.000
34.4	1	7.000	5	1	2.500
35	1	3.000	5.2	1	2.500

APPENDIX 1 Continue

2008-2009

35.1	1	11.500
35.2	1	10.000
35.5	1	2.000
35.7	1	9.000
35.8	1	1.000
36	1	4.000
36.3	2	17.500

Mann-Whitney Test Statistic =9.832
Probability is **0.50** assuming Chi-square
distribution with 10 df

Mann- Whitney Test One-Way Analysis
of Variance for 12 cases
Dependent variable is MIFEMALE
Grouping variable is MAGNESIUM

Group	Count	Rank Sum
5.41	1	6.000
8.74	1	4.000
9.87	1	2.000
12.54	1	8.000
14.56	1	1.000
15.62	1	9.000
18.3	1	10.000
21.08	1	12.000
24.3	2	16.000
30.5	1	3.000
47.4	1	7.000

Mann- Whitney Test Statistic =9.615
Probability is **0.50** assuming
Chi-square distribution with 10 df

5.3	1	9.000
5.7	1	8.000
6.1	1	6.000
6.6	1	1.000
6.8	1	4.000
7.4	1	11.000
7.5	1	10.000

Mann-Whitney Test Statistic =9.109
Probability is **0.52** assuming Chi-square
distribution with 10 df

Mann-Whitney One-Way Analysis of
Variance for 12 cases
Dependent variable is IPFEMALE
Grouping variable is DO

Group	Count	Rank Sum
4.5	2	12.000
4.8	1	4.000
5	1	5.000
5.2	1	1.000
5.3	1	6.000
5.7	1	11.500
6.1	1	11.500
6.6	1	8.000
6.8	1	2.000
7.4	1	7.000
7.5	1	10.000

Mann-Whitney Test Statistic =9.611
Probability is **0.50** assuming Chi-square
distribution with 10 df

2009-2010

Mann-Whitney One-Way Analysis
of Variance for 12 cases
Dependent variable is MIFEMALE
Grouping variable is SALINITY

Group	Count	Rank Sum
34.08	1	5.000
34.2	1	3.000
34.5	1	8.000
34.6	1	7.000
34.9	1	10.000
35.3	1	11.000
35.4	1	1.000
35.5	2	14.000
35.6	3	19.000

Mann-Whitney Test Statistic =6.179
Probability is **0.63** assuming
Chi-square distribution with 8 df

Mann-Whitney One-Way Analysis
of Variance for 12 cases
Dependent variable is MIMALE
Grouping variable is DO

Group	Count	Rank Sum
4.1	1	8.000
5.2	1	3.000
5.3	1	7.000
5.5	2	15.000
5.6	2	13.000
6	1	9.000
6.1	1	1.000
6.4	1	6.000
6.7	1	12.000
7.4	1	4.000

Mann-Whitney Test Statistic =6.923
Probability is **0.65** assuming
Chi-square distribution with 9 df

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