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Original Article

An account on the assemblage of fish larvae in Ponnani estuary, South India

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Abstract: Estuarine environments are one of the most dynamic aquatic ecosystems and serve many important functions in coastal waters. Larval fish dynamics contribute significantly to understanding the ecology of fish populations as they can indicate the spawning-stock biomass and recruitment in adult fish stocks. Initial development stages of fishes are particularly vulnerable and are influenced by physical and biological processes. Hence the present study was aimed to characterize ichthyoplankton assemblages, to evaluate environmental influence in its structure. Ponnani backwater fish larvae assemblages displayed a clear seasonal pattern presenting higher abundances and diversities during warmer months. Throughout the year there is a wide fluctuation in salinity, temperature and primary productivity in these backwaters enabling it to be classified under stressful environment for larval forms of certain economically important marine fishes. A detailed analysis made to study the interaction of selected environmental parameters with ichthyofaunal diversity in Ponnani backwater provided a clear understanding on the influence of these variables on the distribution of marine fish larvae in the region. The results of the present analysis provided a model for the prediction of larval diversity from the prevailing environmental parameters.

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Introduction

Fish communities inhabiting estuaries represent a combination of freshwater, resident and marine species, including many larvae and juveniles. Habitat selection and non-random use of space occupied by these groups are often influenced by abiotic and biotic factors (Whitfield, 1999). The importance of these two factors in driving the spatial and temporal patterns of occurrence of fish in estuaries is still not been investigated in depth. But a variety of abiotic factors have been shown to influence the utilization of estuaries by fishes (Cabral et al., 2001; Martinho et al., 2007). Salinity and turbidity on a spatial scale and temperature on a temporal scale have been regarded as best predictors for estuarine species abundance and spatio-temporal community structure (Bruno et al., 2013). Nonetheless, it has also been suggested that biotic processes, such as food availability, competition and predation, may be important in driving the occurrence of spatial and temporal patterns of fish in estuaries (Akin et al., 2005).

Up to now, only few studies have analysed simultaneously the effect of both biotic and abiotic factors on estuarine fish structures and assemblages (Beck et al., 2001; Martino and Able, 2003; Trevor and Whitfield, 2006; Selleslah and Amara, 2008; Castro et al., 2009). Many authors have also shown that they exhibit strong short and long-term variability, due to environmental fluctuations (Day et al., 1989). Both spatial and temporal studies of fish assemblages are therefore needed to understand fully the functioning of environmental variables in any dynamic ecosystem such as the estuary. Tropical lagoons and estuaries are the subject of an increasing scientific interest because of the expansion of human activities in these areas and the awareness of their economic and ecological importance.

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Estuaries have been the subject of considerable ichthyological research, some of which have focused on the key environmental factors affecting estuarine fish community structure (Elliott et al., 2007). Estuarine fish assemblages have been studied since the late seventies (Whitfield et al., 1994; Blaber, 2000). Estuaries are the focus of attention because of the concentration of large components of the human population in these watersheds, the resulting increasing urbanization of coastal watersheds and concomitantly the alteration and loss of habitats. Investigating climatic effects and climate change trends is an urgent issue all over the world, but even more so in tropical regions where alterations in temperature and precipitation are expected to be more accelerated than the global mean alteration rate (Intergovernmental Panel on Climate Change, 2001). Alterations in temperature and precipitation have the potential to strongly affect the hydrological features that affect fish larvae, such as temperature and currents, and the magnitude of river plumes. It is thus very important to understand how climate and hydrology affect fish larvae movement towards estuarine nurseries. Since each species has different environmental ranges and requirements, different species will be influenced differentially by different variables in the process of estuarine immigration (Vinagre, 2009). Ramya (2010) documented unusual occurrence of larvae and juveniles of economically important marine fish in the Ponnani backwaters. Also prevalence of species formerly rare in the area had increased. These changes in fish distribution correlate to the temperature and salinity preference of the species. Larvae and juveniles of fishes that spawn near the coast prefer slightly lesser temperature and salinity than that in open ocean (Kimura et al., 2010). However, in pursuit for better rearing environment they may migrate to estuaries (Albe, 2005).

The relationship between environmental factors and the distribution of organisms within estuaries has received considerable attention. Because fishes are one of the dominant macrofaunal components of estuarine biota, many studies have focused on their distribution patterns. Central to these studies are the determinations as to what factors, and what levels of those factors, are relevant in defining fish habitat within estuaries (Able, 2005). The knowledge of juvenile fish growth in extreme environmental conditions is a key to the understanding of adaptive responses and to the relevant management of natural populations. Under the above pretext, a detailed analysis on the impact of different environmental variables on the occurrence and distribution of larvae of commercially important marine fishes was studied. The results would provide an in depth knowledge on the environmental dynamics prevailing in the Ponnani backwaters and help fishery biologist to develop models for postulating the impact of the climate change to the ichthyofauna of the region.

Materials and Methods

Study Area: The Ponnani estuarine system is located between 10°46' and 10°48'N and 75°54' to 75°56'E. It is an open estuary that is drained by a tributary of the Bharathapuzha River and drains in to the Arabian Sea at this estuary. The estuarine systems exposed to tides from the Arabian Sea and hence water is brackish almost throughout the year.

Sampling: Sampling was carried out once every month during daylight at high tide, from May to April at three separate stations along the Ponnani backwaters, viz., Veliyancode, Ponnani and Biyyam (Fig.1). Samples were collected by horizontal subsurface tows using plankton net (mesh size 500 mm; mouth diameter: 0.5 m). The samples were then preserved in a 4% buffered formaldehyde seawater solution.

In the laboratory, individuals were identified and counted. Larval identification was done following Leis and Carson-Ewart (2000) and the key provided in www.larvalbase.org. Relative position of the dorsal and anal fin-ray, vertebrae counts, pigmentation patterns shape, number and arrangement of myomeres, and other prominent physical features were used to identify fish larvae (Leis and Carson-Ewart, 2000). Fish larvae were

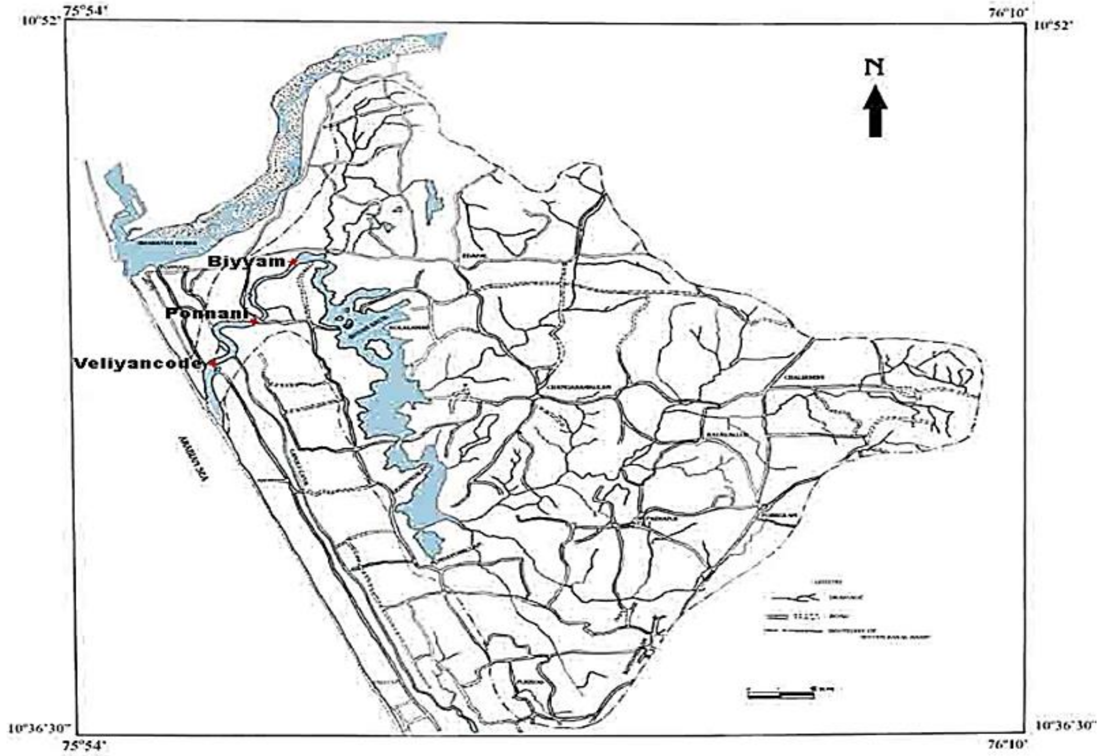

Figure 1. Map of the Ponnani Backwaters showing the stations.

identified under stereomicroscope to family level by using the descriptions of related taxa. After identification, the larvae were photographed and preserved in 70% alcohol. Unidentified larvae were placed in "unknown" category due to the samples were too small to identify and damaged larvae were placed in "incomplete" category.

Water samples from the three stations were collected on a monthly basis from May to April. The water quality parameters recorded on site were temperature, dissolved oxygen, salinity, pH, total suspended solids, and turbidity using portable water and soil analysis kit (EI-Model 191), while nutrients such as nitrate and phosphate were analyzed in lab based on Strickland and Parsons (1972) and measured on Systronics UV-VIS Spectro-photometer. Subsequently from each site phytoplankton and zooplankton samples were also enumerated using Sedgwick Rafter counter or Heamocytometer (APHA, 2005).

Statistical Analysis: The software programmes viz., SPSS (Statistical Product and Service Solutions version17.0) and PRIMER 6 (Plymouth Routines in Multivariate Ecological Research, Version 6.1.9) were used for univariate and multivariate analyses of data. On the base data, descriptive statistics for the biological and hydrographical parameters of each sample were worked out and averaged over the stations. One-way ANOVA, and Duncuns Multiple Range Tests (DMRT) were performed to test the significant differences between means of various parameters recorded from different stations during the three seasons. The results were used to correlate the environmental parameters with biological parameter by backward regression method, in which most statistically insignificant variables were avoided for further analysis. In the next step, two parametric multivariate analyses (factor analysis and regression analysis) were employed to examine the possible influence of environmental variables on the diversity of marine ichthyofauna.

Factor analysis was particularly useful for considering several related random environmental variables simultaneously to identify a new smaller set of uncorrelated variables that accounted for a large proportion of total variants in original variables (Lau and Lane, 2002). In factor analysis, the extracting factors that account for variance is called

Table 1. Mean values of environmental variables during different seasons in the three stations of the Ponnani backwaters.

Environmental Variables	Veliyancode						Ponnani						Biyyam					
	Premonsoon		Monsoon		Post Monsoon		Premonsoon		Monsoon		Post Monsoon		Premonsoon		Monsoon		Post Monsoon	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Temperature Surface (°C)	28.25	0.38	25.60	1.07	27.05	0.41	28.00	0.42	26.95	0.47	27.28	0.75	30.00	1.59	31.18	1.01	28.75	0.61
Temperature Bottom (°C)	26.35	1.34	22.00	0.67	24.05	1.73	25.48	0.49	23.13	0.67	22.70	1.81	25.03	0.83	23.98	0.92	24.10	1.04
Dissolved Oxygen (ppm)	6.95	0.71	6.78	0.38	7.05	0.25	7.28	0.33	6.68	0.29	7.25	0.37	5.98	0.25	5.48	0.64	5.98	0.13
Salinity (ppt)	22.00	2.45	14.00	1.63	18.00	2.94	17.75	0.50	11.75	1.71	15.00	2.58	13.25	2.50	9.00	2.00	10.50	3.00
pH	7.63	0.13	7.40	0.24	7.58	0.17	7.70	0.12	7.83	0.39	7.53	0.28	7.83	0.21	7.85	0.34	7.73	0.10
TSS	4.68	1.45	4.20	1.86	4.18	1.69	5.70	0.73	7.08	0.80	4.53	1.66	3.55	0.76	4.55	0.60	3.50	1.78
Turbidity	14.00	1.83	29.75	17.26	27.75	9.63	14.25	1.71	22.25	5.32	21.00	2.94	15.00	2.58	19.00	1.83	12.00	1.63
Nitrate (ppm)	3.48	1.91	5.28	1.27	5.23	0.68	4.85	1.09	5.90	0.75	4.18	1.24	3.75	0.44	3.90	0.22	3.73	0.36
Phosphate (ppm)	8.35	3.55	6.58	0.50	7.28	3.76	15.20	4.90	9.80	2.42	11.38	5.47	7.95	4.94	10.10	2.30	10.05	3.87

Table 2. Analysis of fish larval assemblage, diversity and evenness in different stations of the Ponnani backwaters.

Parameters		sum of squares	df	mean square	F
Number of Individuals	Between Groups	5130.667	2	2565.333	26.069**
	Within Groups	3247.333	33	98.404	
	Total	8378.000	35		
Number of Species	Between Groups	262.889	2	131.444	24.449**
	Within Groups	177.417	33	5.376	
	Total	440.306	35		
Shannon diversity index	Between Groups	3.236	2	1.618	14.711**
	Within Groups	3.629	33	.110	
	Total	6.865	35		
Simpson evenness	Between Groups	0.084	2	0.042	7.785**
	Within Groups	0.177	33	0.005	
	Total	0.261	35		

**Significant at 0.01% level.

the Eigen value. Therefore higher Eigen value means, higher percentage of variance exhibited by the factor (Wang et al., 2007). Hence the first factors with higher Eigen value was considered for further analysis which will cumulatively contribute much to the total variance. To establish the relationship between the environmental variables and ichthyofaunal diversity, a backward multivariate regression analysis was employed using diversity indices as dependent variable and the factors obtained by factor analysis as independent variables using SPSS v17.0. Principal Component Analysis was adopted for deriving the factors following Varimax rotation with Kaiser Normalization. This was followed by construction of a factor loading matrix to delineate the most prominent environmental factor influencing the distribution of fish fauna in the Ponnani Estuary.

Community Structure: PRIMER 6 for Windows was used for the analysis of community structure and following diversity indices i.e. number of individuals (Abundance; N), total number of species (S), Shannon-Weiner Diversity Index (H’) and Simpson’s Evenness Index (D).

Results

During the present study, 754 fish larvae belonging to 14 different families were identified. However, four larvae could not be identified. The mean values of different environmental variables recorded in the present study are depicted in Table 1. The results indicate a significant difference (*P*<0.01) in all the parameters studied during the three seasons and across the three stations. Table 2 depicts the analysis of variance in the number of individuals and species, mean diversity and evenness of the population. The results indicate that all these parameters varied significantly (*P*<0.01) among the three stations. This implies that the assemblage of fish larvae in Veliyancode, Ponnani and Biyyam were different

Table 3. Duncun's multiple regression test on fish larval assemblage, diversity and evenness in different stations of the Ponnani backwaters.

Variables	Veliancode	Ponnani	Biyyam
Number of Individuals	37.67^{a}	15.00^{b}	10.33^{b}
Number of Species	11.75^{a}	7.42^{b}	5.25^{c}
Shannon diversity index	2.27^{a}	1.80^{b}	1.54^{b}
Simpson's evenness index	0.12^{a}	0.20^{b}	0.24^{b}

Means with similar sets of superscripts are homogenous.

Table 4. Analysis of fish larval assemblage, diversity and evenness in different seasons in the Ponnani backwaters.

Parameters		sum of squares	df	mean square	F
Number of Individuals	Between Groups	6135.500	8	766.938	9.234**
	Within Groups	2242.500	27	83.056	
	Total	8378.000	35		
Number of Species	Between Groups	340.056	8	42.507	11.448**
	Within Groups	100.250	27	3.713	
	Total	440.306	35		
Shannon's diversity index	Between Groups	4.866	8	0.608	8.218**
	Within Groups	1.998	27	0.074	
	Total	6.865	35		
Simpson's Evenness index	Between Groups	0.145	8	0.018	4.246**
	Within Groups	0.115	27	0.004	
	Total	0.261	35		

**Significant at 0.01% level.

and while correlating this result with that of environmental variables, it could be ascertained that the cause of variation should be due to change in the physico-chemical characteristics of the region.

The results also showed that mean values of number of individuals were significantly high (P<0.01) for Veliyancode indicating a rich density of marine fish larvae compared to other two stations. Similarly, the number of species in Veliyancode showed significantly higher representation. Veliyancode had highest diversity of fish larvae followed by Ponnani and Biyyam. In contrast, the Simpson's Evenness index was least in Veliyancode which indicate that the probability of a single being sample continuously is less. This also indicate that the larval diversity in Veliyancode is significantly better than the other two stations thereby showing that this part of the estuary is most probable to have more marine fish larvae than any other part of the Ponnani backwater.

The results of DMRT indicated that in terms of number of individuals, Shannon's diversity and Simpson's evenness indices, Ponnani and Biyyam, were grouped together since they showed similar characteristics (Table 3). However, Veliyancode, which was more prone to salinity incursion distinctly stood out from rest of the stations in all the parameters analysed. This means that the physic-chemical characteristics prevailing in this region is unique and favourable for better diversity and number of larvae of marine fishes. In the second set of analysis, seasons (premonsoon, monsoon and post monsoon) was kept as the variable and data was pooled accordingly for distinguishing variations in larval assemblage. The results also indicated that even season-wise has been a significant (P<0.01) variation in the number of individual, species, diversity and evenness in Ponnani backwaters (Table 4). Hence, based on the above results, it can be confirmed that the environmental parameters correlates have a direct bearing on the distribution of ichthyofauna in backwaters of Ponnani. Figures 2 and 3 denotes the mean values of number of individuals and species respectively of marine fish

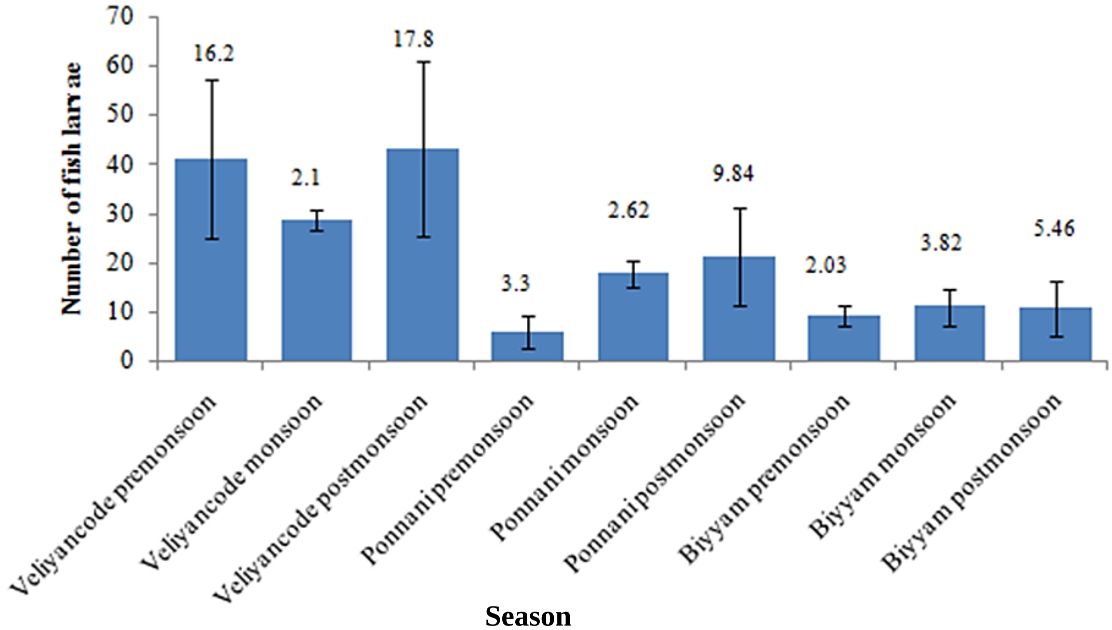

Figure 2. Variation in the number of individuals during different seasons.

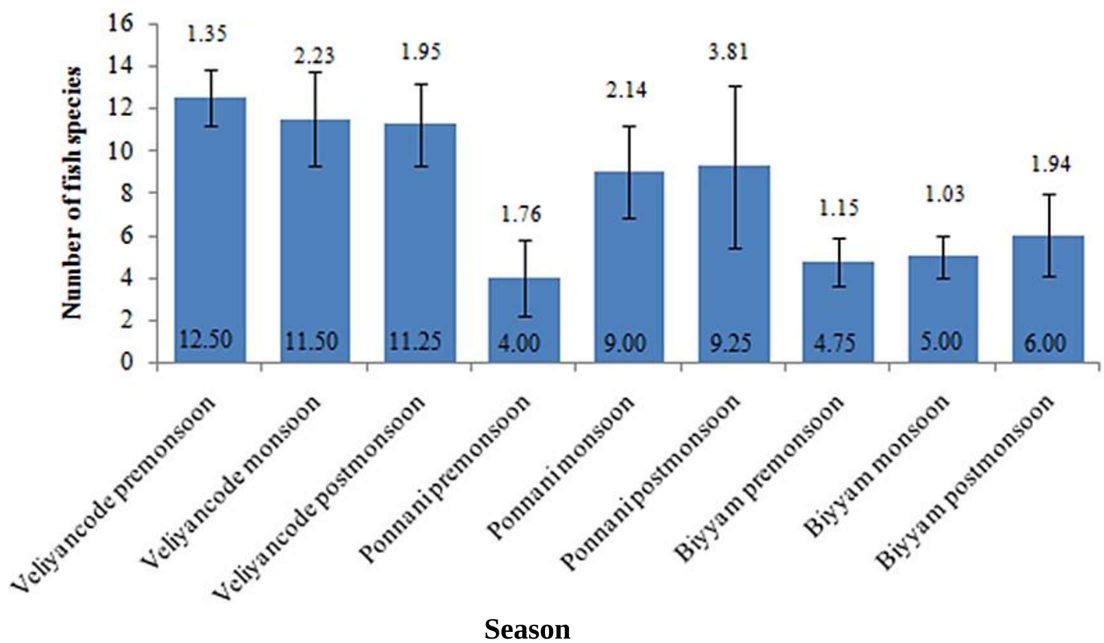

Figure 3. Variation in the number of species in different seasons.

larvae encountered during different seasons in the three stations. The results show a clear dominance of number of individual during post monsoon season in all the stations. While analysing the pattern of distribution of fish species during different seasons, it could be seen that maximum representation in Veliyancode was during premonsoon periods and in quite contrary the numbers were comparatively high during post monsoon season in Ponnani and Biyyam.

The Shannon's diversity index also followed a similar trend as seen for number of species (Fig. 4). However, an interesting observation was reduced values of marine fish diversity during premonsoon season in Ponnani. In spite of being a comparatively lesser saline environment, Ponnani estuary showed good representation of marine fish larvae similar to that seen in Veliyancode during monsoon and post monsoon seasons. Simpson evenness index on the

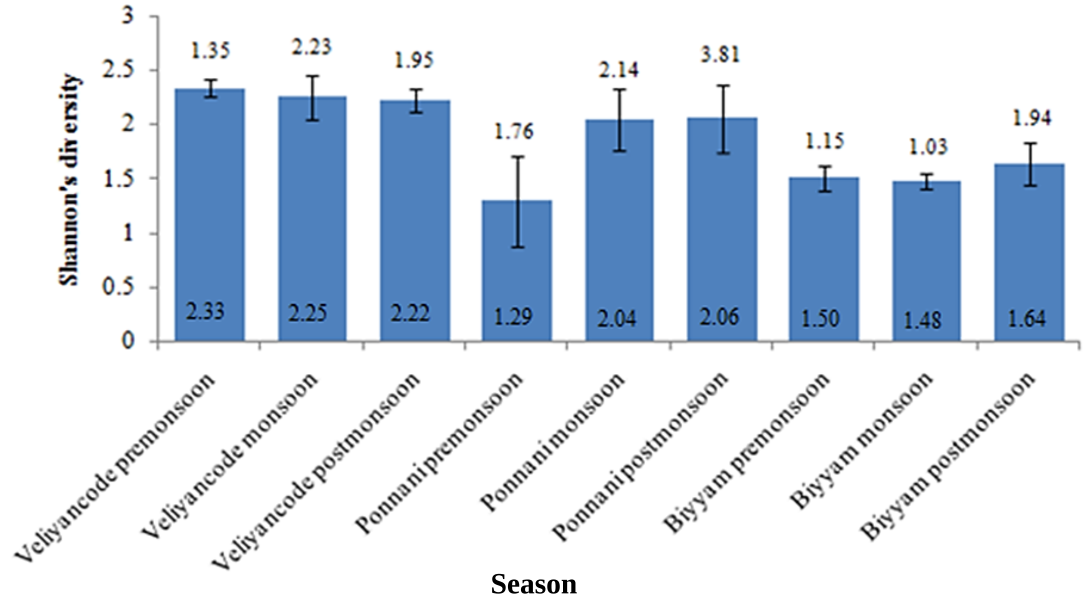

Figure 4. Seasonal-wise variations in the Shannons's diversity indices.

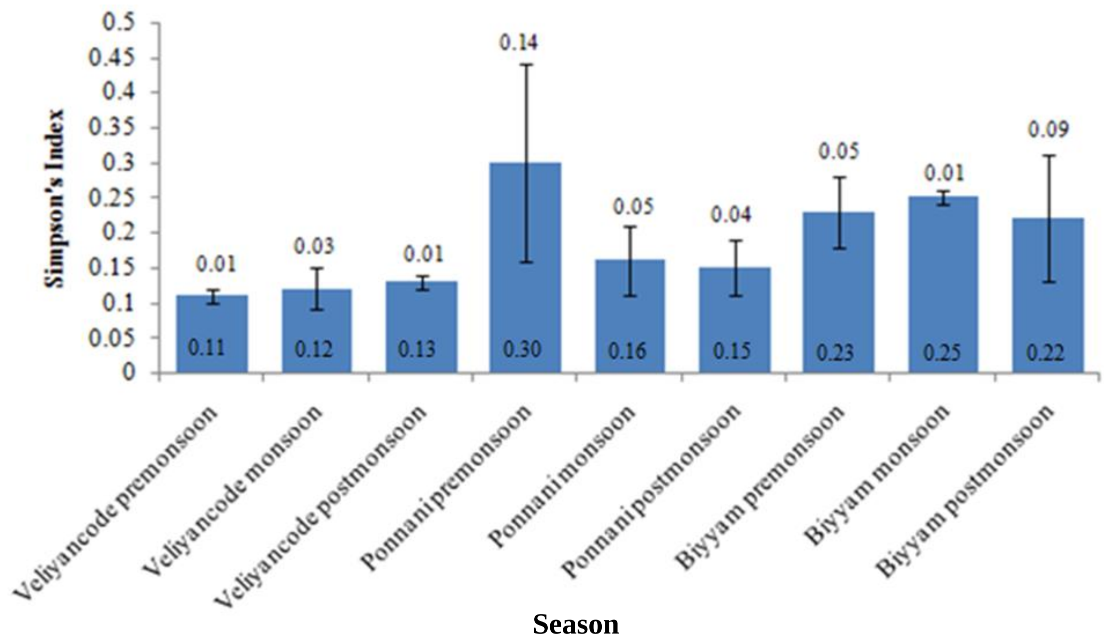

Figure 5. Seasonal-wise variations in the Simpson's evenness.

contrary was high during premonsoon in Ponnani (Fig. 5). The results undoubtedly emphasized that chances of finding different families of marine fish larvae was high in Veliyancode station during all seasons. Similar results were noticed for monsoon and post monsoon periods in Ponnani as well. This indicates that except for Biyyam the other two stations had fairly good population of marine ichthyofauna throughout the year. In Biyyam, the number of individuals (N), number of species (S), Shannon's diversity (H') and Simpson evenness remained rather same during three seasons (Table 5). This means that the environmental perturbation in this station is comparatively less than the other two stations, thereby reflecting a similar kind of population throughout study period.

Factor analysis of environmental variables collected from the three stations of Ponnani backwater pooled together during the three seasons showed that the Eigen values for the first four factors

Table 5. Duncun's multiple regression test on fish larval assemblage, diversity and evenness in different seasons of the Ponnani backwaters.

Variables	Veliancode			Ponnani			Biyyam		
	Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
Number of Individuals	$41.00^{d,e}$	$28.75^{c,d}$	43.25^{e}	6.00^{a}	$17.75^{a,b,c}$	$21.25^{b,c}$	$9.25^{a,b}$	$11.00^{a,b}$	$10.75^{a,b}$
Number of Species	12.50^{c}	$11.50^{b,c}$	$11.25^{b,c}$	4.00^{a}	9.00^{b}	9.25^{b}	4.75^{a}	5.00^{a}	6.00^{a}
Shannon diversity index	2.33^{c}	2.25^{c}	2.22^{c}	1.29^{a}	$2.04^{b,c}$	$2.06^{b,c}$	1.50^{a}	1.48^{a}	$1.64^{a,b}$
Simpson's Evenness index	0.11^{a}	$0.12^{a,b}$	$0.13^{a,b}$	0.30^{e}	$0.16^{a,b,c,d}$	$0.15^{a,b,c}$	$0.23^{c,d,e}$	$0.25^{d,e}$	$0.22^{b,c,d,e}$

Means with similar sets of superscripts are homogenous.

Table 6. Environmental variables based on eigen value, percentage of variance and cumulative percentage.

Component	Extraction Sums of Squared Loadings		
	Eigen value	Percentage of Variance	Cumulative percentage
1	3.119	28.352	28.352
2	2.280	20.728	49.080
3	1.774	16.128	65.207
4	1.034	9.400	74.608

Table 7. Factor loading matrix for different environmental variables observed in the Ponnani backwaters.

Environmental/ Biological Parameters	Component 1	2	3	4
Surface water temperature	-0.744	0.122	0.474	0.017
Bottom water temperature	-0.319	0.83	-0.015	0.162
Dissolved oxygen	0.758	0.398	-0.287	0.199
Salinity	0.271	0.667	-0.494	0.331
pH	-0.319	0.308	0.634	0.104
Total Suspended Solids	0.476	-0.046	0.626	0.333
Turbidity	0.415	-0.498	0.144	0.616
Nitrite	0.61	-0.227	0.031	-0.381
Phosphate	0.167	0.586	0.407	-0.033
Phytoplankton abundance	0.577	0.48	0.194	-0.452
Zooplankton abundance	0.763	-0.047	0.452	-0.077

were 3.119, 2.280, 1.774 and 1.034 (Table 6). F1, F2, F3 and F4 together accounted for 74.61% of the total variance. F1 which contributed to 28.35% of the total variance was related to surface water temperature, dissolved oxygen and abundance of zooplankton, F2 explained 20.73% of the total variance and was related to bottom water temperature and salinity, F3 accounted 16.13% of the total variance and was related to pH and Total Suspended Solids (TSS), and, F4 supported only 9.40% of the total variance related to turbidity (Table 7).

The relationship of marine fish larval diversity indices to the factors, worked out for different seasons, was established using stepwise regression analysis. Since the environmental parameters and diversity indices showed significant ($P<0.01$) variation with respect to season and region, regression equations were developed to correlate each ichthyofaunal diversity indices and related environmental variables for the study area by pooling the three stations and seasons.

Abundance of marine fish larvae (N):

$$N=21.00-7.512\times F2+5.035\times F3-4.961\times F1$$

The results suggest that F2 and F1 showed significant negative relationship with abundance of marine fish larval communities in Ponnani backwaters, while F3 had a positive bearing on the abundance of marine ichthyoplanktons. Hence environmental variables positively favouring better abundance of fish larvae were pH and TSS, while temperature, DO and salinity negatively influenced this variable.

Species abundance (S):

$$S=8.139-1.872\times F2+1.317\times F3+0.979\times F4$$

Species abundance was instead positively favoured by factors F3, F4 and negatively influenced by F2. A significant finding of the study was that both bottom water temperature and salinity were the deciding factors for larval assemblage in Ponnani backwaters.

Shannon-Wiener diversity index (H'):

$$H'=2.442-0.314\times F2+0.270\times F3$$

Similar to earlier results, F2 was the chief contributing factor for this variable. Here only two factors (F2 and F3) contributed to the diversity indices. Hence F2 could be considered as a limiting factor responsible for not only the population size but also the diversity of marine fish larvae inhabiting backwaters of Ponnani.

Simpson's evenness (1-λ):

$$1-\lambda= 0.186+0.039\times F2$$

Evenness was influence by only factor F2. Hence there is a greater role of climatic variables such as temperature and salinity in an estuarine environment in determining the evenness of population available at a particular locality at a specific period of time.

Discussions

The present investigation was conducted to explore the interactions between environmental factors with abundance and diversity of marine fish larvae in three regions of the Ponnani backwaters. The results underline the fact that there is both spatial and temporal distribution of marine fish larvae in Ponnani backwaters, the environmental parameters have a direct role. Hence delineating the most salient environment factor influencing this distribution would throw more light on to the dynamics of larval assemblage. It could also be a combination of factors that influence the larval dispersal. Hence, an in depth analysis based on stations and seasons was attempted to elucidate whether a specific set of parameters are responsible for the larval diversity in Ponnani backwaters. Among the three stations, Veliyancode throughout the study period showed high proportions of marine ichthyofana both adult and larval forms. Both in terms of number of individuals and families represented this station stood apart from other two. Being in close proximity to the Arabian Sea and with salinity gradient much narrow, this harbours the richest diversity of marine ichthyofauna in the region. Although in terms of Shannon's diversity and Simpson's evenness scales, the place shared a unique position with Ponnani estuary, on closer observation it could be seen that typical marine fauna were more concentrated in Veliyancode whereas Ponnani was dominated by marine and estuarine fish fauna. Hence in a nutshell it could be consolidated that although the three station were in close proximity to each other, the environmental regime featuring in the region was quite distinct, which ultimately reflected in the diverse pattern of fish fauna prevalent in the three stations. Impact of monsoon was more pronounced in Biyyam which has been receiving greater drainage of freshwater from Bharathapuzha River. This is why lesser marine species were documented from the region. This also means that salinity does play a major role in the distribution of fish in Ponnani backwaters.

The statistical evidence suggested that there exist high spatial heterogeny among the relationship between environmental variables and fish larval diversity. Although an array of environmental factors indulge on the biotic component of an aquatic system, very few affect them regularly. However, if any such limiting factor is observed, they either act singly or in combinations with other minor entities to produce a change in the ecological structure. In the present study, although there were eight factors that influence the faunal assemblage in the estuary only two variables were responsible for most of the changes. Hence it can reasonably be concluded that the temporal and spatial distribution of marine fish larvae in Ponnani backwaters is profoundly influenced by the physico-chemical conditions prevailing in a particular area. This makes biodiversity assessment more challenging in a dynamic environment such as an estuary where there are more than a dozen factors that continuously interact with the organisms. Marine fish larvae hence are easy target of even a slightest change in these environmental parameters and which could deleteriously affect their recruitment and further survival. Understanding the influence of environmental predictors would in turn be used as an effective tool for developing appropriate policies in the conservation of estuaries and fish fauna available in it.

Conclusion

The present study suggests that there exist a high spatial heterogeneous relationship between environmental variables and ichthyofaunal diversity. In the present study although there were eight factors that influence the faunal assemblage in the estuary, however only temperature and salinity were the variables responsible for most of the changes. Hence the major limiting factor for all the diversity indicators was temperature and salinity. The degree to which they contribute any variation depends on the influence of other factors either acting synergistically or antagonistically. Whatever may be the case the present study clearly revealed the influence of environmental variables on the fish fauna of the Ponnani backwaters. It would thus confirm the earlier finding of spatial and temporal variations that salinity along with bottom water temperature play a vital role in the survival and distribution of marine fish larvae in the Ponnani backwaters. The present study paves way for an in depth analysis of all available environment parameters acting upon the fish fauna of Ponnani estuary to ascertain their role in determining the availability and distribution of commercially important marine fish in the backwaters of Ponnani. Only then shall it be clearer how each environmental correlates contribute to the fish diversity of the region.

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