Oceanography & Fisheries
Open access Journal
ISSN: 2476-0536

Juniper PUBLISHERS key to the Researchers

Research Article
Volume 12 Issue 2 - August 2020
DOI: 10.19080/OFOAJ.2020.12.555833

Oceanogr Fish Open Access J

Habitat Ecology and Biological Characteristics of a Hypersaline Ciliate, *Fabrea salina* from Solar Salterns of Mumbai Coast, India

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Submission: April 06, 2020; **Published**: August 03, 2020

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Abstract

The ecology of a hypersaline ciliate, *Fabrea salina* was studied in two saltpans along the Mumbai coast, India. There was an apparent trend of its seasonal abundance being maximum (up to 58 x 10^3 cells L^{-1} in May) during late post- monsoon to summer months and complete disappearance during monsoon period. Being the most dominant species in microzooplankton community, it had an average annual density of 18 x 10^3 cells L^{-1}. It flourishes well under higher temperature (30-39ºC) and salinity (40-150 ‰) conditions. Among phytoplankton, *Dunaliella* was the dominant one with a highest density of 58 x 10^3 cells mL^{-1} in April, followed by *Chlorella* with up to 42 x 10^3 cells mL^{-1}, in March. The ANOVA test for physical and chemical variables has revealed significant difference (P=0.05) in their values in different months. Except in water temperature and NO_2-N, no significant difference was observed at various stations as the case with phytoplankton and zooplankton. There was strong positive correlation of *Fabrea* with water temperature (r=0.866, 0.801), salinity (r=0.966, 0.957), total alkalinity (r=0.717, 0.729) and PO_4-P (r=0.750, 0.897) while negative correlation with water depth (r=-0.767, -0.757) and pH (r=-0.086, -0.411). *Fabrea* varies widely in its total length (60-600 µm) and cyst diameter (70-180 µm). The average length of body cilia is 12 µm and the width of each adoral zone of membranelle (AZM) is 10 µm.

Kewords: *Fabrea salina;* Hypersaline ciliate; Solar salterns; Ciliate ecology

Introduction

Ciliates are the most specialized and perhaps most widely distributed and diverse group of protozoa having representatives in virtually all kinds of freshwater to marine environments, often in extremely high densities [1]. They form an important component of estuarine as well as coastal marine ecosystems as they feed upon bacteria and in turn serve as food for metazoans [2,3]. However, the ecology and biology combined with factors controlling the distribution of protists in tropics have received very little attention [4]. Many hypersaline environments are inimical to macroscopic life but are the preferred habitats of a variety of microorganisms. The heterotrichous ciliate, F. salina , reported from several diverse environments such as salt marshes, hypersaline lakes and solar salterns [5-7], has received much attention in the recent years primarily due to its potentiality as live food source for maricultural purposes [8-11] and also as an experimental animal in basic research of applied value in eukaryotic microbiology [12-16]. Though fairly a good amount of literature is available on the hydrobiology of estuaries and backwaters in India, the information on the plankton ecology in inland saline lakes and solar salterns is very scanty. The present study deals with the ecology of *F. salina* in two saltpans near Mumbai, West Coast of peninsular India, for a period of one year, January 1998 to December 1998.

Study Area

The study areas, Mira Road and Bhayandar saltpans, the parts of Thane District, Maharashtra, India, are located at 19º16'N Lat & 72º51'E Long and 19º19' Lat & 72º51'E Long, respectively. The former relates to Manori creek while the later with Bassein creek, along the Mumbai coastline. Two sampling stations in each saltpan, denoted as MR_1 & MR_2 at Mira Road and BH_1 & BH_2 at Bhayandar were selected in the present study. The region has typical tropical climate.

Methods

Phytoplankton and Zooplankton

For phytoplankton analysis, one-liter water was directly collected whereas zooplankton samples were taken by filtering

50 L of water through plankton net of 40 µm mesh size. The samples were preserved with Lugol's solution. After three days of stagnation, the phytoplankton samples were concentrated to 100 mL volume by decanting the supernatant. Except *Dunaliella* that was counted by haemocytometer, all the plankton were enumerated using Sedgwick-Rafter cell counter (50mm x 20mm x 1mm). For each month, the average density of *Fabrea*, *Dunaliella* and *Chlorella* were taken for statistical purposes.

Hydrological and Soil-Quality Parameters

Both ambient and water temperatures were measured using thermometer with 0.1ºC accuracy while water depth by a meter scale. Salinity and pH were recorded at the site using Salinity Refractometer (S/Mill- E, Atago) and portable pH meter (Model No. E Merck 325). Other water quality parameters such as dissolved oxygen, dissolved free carbon dioxide, total alkalinity, ammonium- nitrogen (NH_4^+-N), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N) and phosphorus (PO_4-P) were analyzed following Standard Methods (APHA, 1992) monthly. The values of various parameters obtained at both the stations of each saltpan were summed up and average values are used in data analysis. The soil-quality parameters viz. percentage of sand, silt, clay, organic carbon, organic matter and total nitrogen and phosphorus (mg/100 g of soil sample) were analyzed following Ghosh et al. [17] during pre- monsoon, monsoon, and post-monsoon periods.

Encystment and Excystation

To validate the existence of *F. salina* in encysted form during the periods of non- availability of its free-swimming trophozoites in nature, the sun-dried scum- mat with some soil of salt-pans was immersed at 5 g L^{-1} in saline water (2L) of six different salinities i.e. 30, 40, 50, 60, 80 and 100 ‰ provided with mild aeration. For encystment, the salinity of culture medium (5 L) was raised gradually from 65 to 110 ‰. After harvesting, the cysts were subjected to hatching under different salinities as indicated above. These experiments were carried out at ambient and water temperature of 34±1ºC and 31±1ºC, respectively.

Results

Phytoplankton and Zooplankton

Phytoplankton and zooplankton were in abundance during pre- and post- monsoon months while their density was quite low in monsoon periods (Table 1-4). Zooplankters were completely absent during July and August months. *Dunaliella*, the most abundant species noted, was with a maximum density of 57 x 10^3 cells mL^{-1} during April. *Chlorella*, the second largely available plankton, had the highest density of 40 x 10^3 mL^{-1} in March. This was followed by the occurrence of *Nitzschia* sp., *Navicula* sp., *Anabaena*, *Oscillatoria* and *Rhizosolenia*.

Table 1: Phytoplankton composition at different stations of Mira Road saltpan (No./ml; values are x10^3).

Month	*Dunaliella* sp.		*Anabaena* sp.		*Chlorella* sp.		*Oscillatoria* sp.		*Nistzschia* sp.		*Navicula* sp.		*Rhizosolenia* sp.	
	MR1	MR2	MR1	MR2	MR1	MR2	MR1	MR2	MR1	MR2	MR1	MR2	MR1	MR2
Jan	7.5	6.4	-	-	17	17.6	1.5	1.8	2	2.4	1.5	1.2	-	-
Feb	11	13	0.44	0.29	34	37	2	1.8	2.3	2.1	2.2	2	-	-
March	34	38	-	-	42	38	0.42	0.7	-	-	-	-	-	-
April	56	58	0.62	0.5	35	32	1.2	1	-	-	0.5	0.36	-	-
May	52	55	0.28	0.38	-	-	-	-	-	-	-	-	-	-
June	-	-	-	-	-	-	-	-	-	-	-	-	-	-
July	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aug	-	-	-	-	-	-	-	-	-	0.4	-	-	0.72	0.6
Sept	-	-	0.4	-	-	-	-	-	1.6	1.4	1.2	0.8	0.81	0.63
Oct	6	7.5	10	11.2	-	-	-	-	14.4	16	4.2	4.5	1.2	1
Nov	8.2	8	0.26	0.5	13	11	0.6	0.64	2.5	2.8	2.1	2.4	-	-
Dec	11	9.7	1	0.8	16	14	1	0.82	2	2.2	0.56	0.42	-	-

Table 2: Phytoplankton composition at different stations of Bhayandar saltpan (No./ml; values are x10^3)

Month	*Dunaliella* sp.		*Anabaena* sp.		*Chlorella* sp.		*Oscillatoria* sp.		*Nistzschia* sp.		*Navicula* sp.		*Rhizosolenia* sp.	
	BH1	BH2	BH1	BH2	BH1	BH2	BH1	BH2	BH1	BH2	BH1	BH2	BH1	BH2
Jan	6	4.5	-	-	26	24	0.8	0.65	1.2	1	0.68	0.42	-	-
Feb	8	7.5	0.5	-	28	32	0.52	0.6	-	-	-	0.24	-	-

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March	28	32	0.24	0.26	18	16.5	0.56	0.74	-	-	-	-	-	-
April	54	49	-	-	-	-	0.8	1.2	-	-	-	-	-	-
May	46	43	-	-	-	-	-	-	-	-	-	-	-	-
June	-	-	-	-	-	-	-	-	-	-	-	-	-	-
July	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aug.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sept	-	-	-	-	-	-	-	-	1.2	1.4	-	-	1.6	2
Oct	-	-	8	6.5	-	-	-	-	8.6	7.4	2.3	2	1.8	1.5
Nov	4.6	3.8	0.22	-	16	20	0.5	0.74	1.5	1.7	0.5	0.62	-	-
Dec	6	5.4	-	-	22	19	0.45	0.6	0.55	0.6	-	-	-	-

Table 3: Zooplankton composition at Mira Road saltpan (No./l; values are x10^3)

Month	*Fabrea salina*		*Brachionus*		*Artemia* nauplii		*Folliculina*		*Euplotes*	
	MR_1	MR_2	MR_1	MR_2	MR_1	MR_2	MR_1	MR_2	MR_1	MR_2
Jan	12	15	8	10	1	3	-	-	2	-
Feb	18	20	6	5	1	-	-	-	4	1
March	44	38	-	-	-	-	-	-	-	2
April	56	53	-	-	2	-	-	-	-	-
May	58	52	-	-	-	2	-	-	-	-
June	6	2	4	-	-	-	-	-	-	-
July	-	-	-	-	-	-	-	-	-	-
Aug	-	-	-	-	-	-	-	-	-	-
Sept	-	-	2	-	-	-	4	2.6	-	-
Oct	4	3	-	8	-	-	12	10	-	-
Nov	8	11	-	-	-	1.5	-	-	4	2
Dec	14	16	-	2	-	-	-	-	8	5

Table 4: Zooplankton composition at Bhayandar saltpan (No./l; values are x10^3)

Month	*Fabrea salina*		*Brachionus*		*Artemia* nauplii		*Folliculina*		*Euplotes*	
	BH_1	BH_2	BH_1	BH_2	BH_1	BH_2	BH_1	BH_2	BH_1	BH_2
Jan	14	12	6	9	2	-	-	-	-	-
Feb	24	27	8	4	-	4	4	3	-	-
March	42	36	-	-	3	1	2	-	-	-
April	52	47	-	-	-	-	-	-	-	-
May	44	48	-	-	2	-	-	-	-	-
June	8	5	11	8	-	-	-	-	-	-
July	-	-	-	-	-	-	-	-	-	-
Aug	-	-	-	-	-	-	-	-	-	-
Sept	-	-	-	2	-	-	-	-	-	2
Oct	-	3	-	-	-	-	-	-	-	-
Nov	12	15	4	4	-	-	6	2	12	16
Dec	16	12	1.6	-	1.6	-	12	9	-	-

Physical and chemical variables

The highest water temperature (39.0ºC) was recorded at Bhayandar saltpan in May. The water pH was alkaline throughout the year, varying from 8.0 to 8.6. Salinity was very low during monsoon months, varying from nil to 16 ‰ contrary to its higher values (up to152 ‰) prevailing during late post- monsoon and pre- monsoon months. The levels of total alkalinity were generally low during monsoon months being 100 mg L^{-1} in July. The low

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DO levels (1.4- 3.4 mg L^{-1}) were recorded during pre- and post-monsoon months. Generally, the dissolved free carbon dioxide was nil throughout the year. The values of NH_4^+-N, NO_2-N, NO_3-N and PO_4-P were less during monsoon months and higher in post-monsoon periods. There was no wide variation in soil quality parameters at both the saltpans (Table 5,6). The ANOVA has revealed significant difference (P=0.05) in the values of all the 12 physical and chemical variables studied in various months. In addition, water temperature and NO_2-N values showed significant difference at various stations too. There was strong positive correlation of *Fabrea* with water temperature, salinity, total alkalinity, and PO_4-P while negative correlation with water depth and pH (Table 7,8).

Table 5: Soil-quality parameters of Mira Road saltpan.

Parameter	Pre monsoon		Monsoon		Post monsoon	
	MR_1	MR_2	MR_1	MR_2	MR_1	MR_2
Sand (%)	35.0	35.4	37.2	37.3	36.0	36.5
Silt (%)	43.6	43.4	42.8	42.4	43.5	42.8
Clay (%)	21.4	21.2	20.0	20.3	20.5	20.7
Organic carbon (%)	0.75	0.72	0.50	0.55	0.61	0.64
Organic matter (%)	1.293	1.241	0.862	0.948	1.051	1.103
Total nitrogen (mg/100g of soil sample)	75	73	50	55	60	63
Phosphorous (mg/100g of soil sample)	4.3	4.2	4.0	4.0	4.1	4.4

Table 6: Soil-quality parameters of Bhayandar saltpan.

Parameter	Pre monsoon		Monsoon		Post monsoon	
	BH_1	BH_2	BH_1	BH_2	BH_1	BH_2
Sand (%)	34.8	35.2	37.0	37.2	35.6	35.8
Silt (%)	42.6	43.0	42.0	42.6	43.0	43.5
Clay (%)	22.6	21.8	21.0	20.2	21.4	20.7
Organic carbon (%)	0.81	0.74	0.59	0.53	0.66	0.60
Organic matter (%)	1.396	1.276	1.017	0.914	1.138	1.034
Total nitrogen (mg/100g of soil sample)	82	74	58	53	66	61
Phosphorous (mg/100g of soil sample)	4.5	4.4	4.0	4.1	4.2	4.2

Table 7: Statistical analysis of physical & chemical variables and biological studies at Mira Road saltpan.

Physico-chemical parameter				*F. salina*		*Dunaliella*		*Chlorella*	
	Min.	Max.	Mean ± Std. dev.	r	t	r	t	r	t
Temperature (ºC)	25.8	38.4	29.679±4.259	0.866	2.377	0.920	2.806	0.195	3.804
Depth (cm)	31.85	68.95	47.008±13.715	-0.767	3.107	-0.740	3.332	-0.602	4.510
PH	8.0	8.6	8.417±0.180	-0.086	-1.589	0.010	-1.240	-0.483	-0.959
Salinity (‰)	1.0	121.0	50.083±44.544	0.966	4.435	0.928	4.497	0.663	3.578
Alkalinity (mg/l)	100.0	230.5	150.417±33.819	0.717	19.259	0.689	18.921	0.137	13.519
DO (mg/l)	2.5	8.0	4.775±1.953	-0.784	-2.045	-0.715	-1.726	-0.747	-1.615
DCO_2 (mg/l)	0.0	1.0	0.350±0.458	0.292	-2.959	0.242	-2.593	0.170	-2.764
NH_4^+-N (mg/l)	0.145	1.315	0.549±0.425	0.670	-2.947	0.643	-2.580	0.234	-2.723
NO_2-N (mg/l)	0.029	0.149	0.096±0.035	0.846	-2.987	0.810	-2.625	0.644	-2.811
NO_3-N (mg/l)	1.170	2.900	1.783±0.541	0.688	-2.749	0.721	-2.386	0.536	-2.478
PO_4-P (mg/l)	0.045	0.650	0.371±0.220	0.750	-2.960	0.682	-2.594	0.492	-2.606

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Table 8: Statistical analysis of physical & chemical variables and biological studies at Bhayandar saltpan.

Physico-chemical parameter				*F. salina*		*Dunaliella*		*Chlorella*	
	Min.	Max.	Mean± Std. dev.	r	t	r	t	r	t
Temperature (ºC)	26.35	38.7	30.529±4.323	0.801	3.014	0.906	4.231	-0.357	5.275
Depth (cm)	28.55	66.4	44.963±11.313	-0.757	3.423	-0.676	4.089	-0.284	6.669
PH	7.9	8.9	8.342± 0.253	-0.411	-1.603	-0.359	-0.752	-0.222	-0.258
Salinity (‰)	1	150	53.667±50.864	0.957	3.729	0.889	4.043	0.263	3.135
Alkalinity (mg/l)	104	264	147±42.780	0.729	14.044	0.717	14.502	-0.03	10.669
DO (mg/l)	1.4	8.1	4.850± 2.330	-0.885	-2.122	-0.739	-1.282	-0.492	-1.153
DCO_2 (mg/l)	0	2.4	0.317± 0.711	0.581	-3.291	0.596	-2.298	-0.068	-2.59
NH_4-N (mg/l)	0.08	1.26	0.531± 0.361	0.591	-3.215	0.49	-2.228	0.079	-2.547
NO_2-N (mg/l)	0.033	0.182	0.121± 0.055	0.821	-3.264	0.697	-2.288	0.531	-2.669
NO_3-N (mg/l)	1.195	2.64	1.758± 0.443	0.461	-2.979	0.359	-1.996	0.442	-2.219
PO_4-P (mg/l)	0.045	0.94	0.345± 0.262	0.897	-3.255	0.821	-2.267	0.171	-2.606

Population of *Fabrea salina*

F. salina was the most dominant species in microzooplankton community. Its density varied from zero in monsoon months to 55 x 10^3 cells L^{-1} in May. The population abundance was in strong correlation with salinity, temperature, alkalinity, water-depth, DO and NO_2-N. *Fabrea* flourishes well under the higher temperature (30-39ºC) and salinity (40-150 ‰) conditions. *Dunaliella* acts as natural food for *Fabrea*. The bloom of *Dunaliella* was noticed during March to May with the concurrent abundance of *Fabrea*.

Cyst Hatching

It is evident that 40 to 50 ‰ salinities are suitable for cyst hatching. No significant hatching occurred beyond 60 ‰ salinity.

Discussion

Phyto and Zooplankton

Dunaliella salina has best growth in 120 ‰ salinity with a tolerance limit of 350‰ [18]. Like *Dunaliella*, diatoms are also ubiquitous inhabitants of hypersaline environments, but they never appear to dominate. The present findings are in conformity with the occurrence of diatoms in solar salterns having salinity up to 129 ‰ in the Great Salt Lake [19]. *Nitzschia* sp. and *Navicula* sp. are represented in all these aquatic environments. The probable factors restricting the eukaryotic algae from many hypersaline environments include their inability to osmoregulation under prevailing conditions and to assimilate nutrients that may be scarce coupled with periodic habitat desiccation.

Physical and Chemical Variables

The saltpans are exposed typically to a wide range of environmental stress and perturbations. On solar heating of brines having halobacterial colouration, a maximum temperature of 46ºC is attained by the densely colored brine, while the clear brines could reach up to 39ºC [20]. Not only do gases diffuse more slowly as brine density increases, the capacity to hold them also becomes poor. Further the low DO levels in the present study were also probably due to bacterial consumption of oxygen that diffuses from the atmosphere or produced by microalga *Dunaliella*. The low pH levels indicate the high levels of CO_2 and alkalinity in water bodies as observed in the present study. The noteworthy trend in salinity values were due to influx of freshwater during monsoon while prevailing higher temperature, excessive evaporation, and low water-depths in the summer months.

Population of *F. salina*

The present study reveals great ability of *Fabrea* to withstand wide ranges of environmental variables. Its better growth has been obtained at 6 x 10^6 and 8 x 10^6 *Dunaliella* cells mL^{-1} [9]. As observed, in solar salterns, it feeds voraciously upon *Dunaliella* cells (Figure 1). However, it appeared that *Fabrea*, in extremely saline conditions (>240 ‰) when *Dunaliella* is not available, survives on halobacterial and it subsists on bacteria during food scarcity [21]. In marine planktonic realm, nearly all phytoplankton produced are consumed, primarily by microzooplankton [22]. Protists are capable of sensing the biochemical properties of their prey cells [23,24], and both ciliates and flagellates have been seen to feed preferentially on more nutritious phytoplankton species [25,26] indicating why *Dunaliella* is preferred by *Fabrea*. *Fabrea* disappears from saltpans in monsoon months as it does not thrive well in brackish water. It forms cyst and resumes normal active form and life activities on the return of suitable conditions during mid of November [9].

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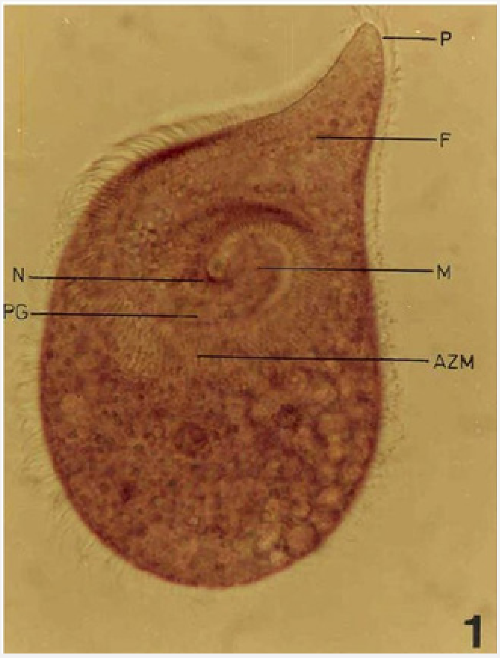

Figure 1: *Fabrea salina* (x40); adoral zone of membranelles (AZM), frontal field (F), mouth area of buccal pouch (M), neuromotorium (N), peristomial groove (PG).

The positive correlation with nitrogen and phosphate is an indication of demand-supply of nutrients to sustain its higher densities in the month of April and May. The optimal production of solar salt requires a well-established balance between primary and secondary producers, with *Artemia* grazing on phytoplankton constitutes the major interaction [27]. *Artemia* also tolerates very high salinities [28]. It is surmised, therefore, that *Fabrea* too contributes to solar salt manufacture. Very sparse, heterogeneous distribution and above all almost vanished populations of *Artemia* from majority of saltpans along the Mumbai coastline make *Fabrea* as a predominant inhabitant of these solar saltworks [29]. The present knowledge of the ecology of *Fabrea* in its natural habitat and effective management practices can be applied for its controlled production on commercial scale in solar salt- beds [30]. The euryplasticity, easy acceptability of a variety of live and inert feeds, short generation period and biochemical composition make *F. salina* as an appropriate animal for studying microbiology of hypersaline environments.

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How to cite this article: Bam Deo P, S G Yeragi. Habitat Ecology and Biological Characteristics of a Hypersaline Ciliate, Fabrea salina from Solar Salterns of Mumbai Coast, India. Oceanogr Fish Open Access J. 2020; 12(2): 555833. DOI: 10.19080/OFOAJ.2020.12.555833

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