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

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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 10³ 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 10³ cells L⁻¹. It flourishes well under higher temperature (30-39°C) and salinity (40-150 ‰) conditions. Among phytoplankton, *Dunaliella* was the dominant one with a highest density of 58 x 10³ cells mL⁻¹ in April, followed by *Chlorella* with up to 42 x 10³ 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 NO₃-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 PO₄-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.

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

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**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 mariculture 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 MR₁ & MR₂ at Mira Road and BH₁ & BH₂ 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 1 mm). For each month, the average density of Fabarea, Dunaliella and Chlorella were taken for statistical purposes.

Hydrological and Soil-Quality Parameters

Both ambient and water temperatures were measured using thermometer with 0.1°C 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 (NH₄-N), nitrite-nitrogen (NO₂-N), nitrate-nitrogen (NO₃-N) and phosphorus (PO₄-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±1°C and 31±1°C, 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). Zooplankton were completely absent during July and August months. Dunaliella, the most abundant species noted, was with a maximum density of 57 x 10³ cells mL⁻¹ during April. Chlorella, the second largely available plankton, had the highest density of 40 x 10³ 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 x10³).

| Month | Dunaliella sp. | Anabaena sp. | Chlorella sp. | Oscillatoria sp. | Nitzschia sp. | Navicula sp. | Rhizosolenia sp. |
|-------|----------------|--------------|---------------|------------------|--------------|-------------|------------------|
|       | MR1 | MR2 | MR1 | MR2 | 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  | -   | -   | -  | -  | -   | -   | -   | -   | -   | -   | 0.4 | -   | 0.72| 0.6 | -   |
| Aug   | -   | -   | -  | -  | -   | -   | -   | -   | 1.6 | 1.4 | 1.2 | 0.8 | 0.81| 0.63| -   |
| Sept  | 6   | 7.5 | 10 | 11.2| -   | -   | -   | -   | 1.44| 1.6 | 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 x10³).

| Month | Dunaliella sp. | Anabaena sp. | Chlorella sp. | Oscillatoria sp. | Nitzschia sp. | Navicula sp. | Rhizosolenia sp. |
|-------|----------------|--------------|---------------|------------------|--------------|-------------|------------------|
|       | BH1 | BH2 | 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| -   | -   | -   |
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     | -    | -     | -    | -    | -    | -    | -    | -    |
| 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 (39.0°C) 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 to 152 ‰) 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
Dissolved oxygen (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 NH₄⁺-N, NO₂⁻-N, NO₃⁻-N and PO₄³⁻-P were less during monsoon months and higher in post-monsoon periods. There was no wide variation in soil quality parameters at both the salt pans (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 NO₂⁻-N values showed significant difference at various stations too. There was strong positive correlation of Fabrea with water temperature, salinity, total alkalinity, and PO₄³⁻-P while negative correlation with water depth and pH (Table 7,8).

Table 5: Soil-quality parameters of Mira Road salt pan.

| Parameter                        | Pre monsoon | Monsoon | Post monsoon |
|----------------------------------|-------------|---------|--------------|
|                                  | MR₁         | MR₂     | MR₁         | MR₂     | MR₁     | MR₂     |
| 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 salt pan.

| Parameter                        | Pre monsoon | Monsoon | Post monsoon |
|----------------------------------|-------------|---------|--------------|
|                                  | BH₁         | BH₂     | BH₁         | BH₂     | BH₁     | BH₂     |
| 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 salt pan.

| Physico-chemical parameter       | F. salina | Dunaliella | Chlorella |
|----------------------------------|-----------|------------|-----------|
| Temperature (°C)                 | Min. 25.8 | Max. 38.4  | Mean ± Std. dev. | r | t | 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      | 50.093±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 |
| DCO₂ (mg/l)                      | 0.0       | 1.0        | 0.350±0.458 | 0.292 | -2.959 | 0.242 | -2.593 | 0.170 | -2.764 |
| NH₄⁺-N (mg/l)                    | 0.145     | 1.315      | 0.549±0.425 | 0.670 | -2.947 | 0.643 | -2.580 | 0.234 | -2.723 |
| NO₂⁻-N (mg/l)                    | 0.029     | 0.149      | 0.096±0.035 | 0.846 | -2.987 | 0.810 | -2.625 | 0.644 | -2.811 |
| NO₃⁻-N (mg/l)                    | 1.170     | 2.900      | 1.783±0.541 | 0.688 | -2.749 | 0.721 | -2.386 | 0.536 | -2.478 |
| PO₄³⁻-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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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\textsubscript{3}⁻. *Dunaliella* flourishes well under the higher temperature (30-39°C) 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 salt pans are exposed typically to a wide range of environmental stress and perturbations. On solar heating of brines having halobacterial colouration, a maximum temperature of 46°C is attained by the densely colored brine, while the clear brines could reach up to 39°C [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\textsubscript{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 10^3 and 8 x 10^3 *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 salt pans 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].
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 salt pans 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* an appropriate animal for studying microbiology of hypersaline environments.

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