Life strategies and aquacultural usability of a hypersaline ciliate, *Fabrea salina*

Ciliates are the most specialized, widely distributed and diverse group of protists. They are an important component of all aquatic ecosystems on earth, from freshwater to almost saturated brines. Their single cell is far more elaborate than any cell of metazoans. The heterotrichous ciliate, *Fabrea salina* had been reported from diverse environments such as salt marshes, hypersaline lakes and solar salterns[1–3]. In India, it was first[4] recorded in 1982 and later[3] in 1998 in solar saltworks along the Mumbai coast. Considering its probable role as live food in marine larviculture practices, its mass culture and analysis of biochemical profile were carried out[5,6]. The present study deals with some biological characteristics such as morphology, cannibalism, phototaxis, salinity tolerance, encystment, excystation and reproduction of *F. salina* and its usability as live food.

(1) The natural stock of *Fabrea* was maintained on the diet of egg-custard (Table 1) in 1 l glass jar. Lugol's solution was used as a general fixative agent while haematoxylin was used for nuclear morphology. (2) As the ciliate is cannibalistic, cannibalism was induced by starving the ciliates for 8 days to study the morphological features of mega-giant forms. (3) To observe phototaxis, its 100 cells were placed in a 15 cm diameter petri dish that was covered with a glass disc, of which the half-part was black painted. One-by-one in five replicates, the ciliates were observed by placing the petri dishes at the light intensity of 250 lumen/m^2, at a distance of 20 cm from a 36 W fluorescent tube. The ciliates in transparent half of the disc were counted at 6 h interval. (4) Salinity tolerance was studied by first acclimatizing the cells to 25 ppt from their culture at 65 ppt, by gradually reducing 5 ppt salinity every two hours. The cells were concentrated with approximately 600 cells/ml. In triplicate, 1 ml of it was transferred to seven small beakers with 50 ml water of different salinities, i.e. 50, 75, 100, 125, 150, 175 and 200 ppt. Desired salinity was achieved by using either distilled water or freshly harvested crude salt, as required. (5) Encystment was induced by gradually raising the culture salinity from 65 to 110 ppt. After harvesting, 100 cysts (in triplicate) were subjected to hatching under six salinity regimes as 30, 40, 50, 60, 80 and 100 ppt, with mild aeration, at ambient and water temperature of 34 ± 1°C and 31 ± 1°C respectively. Saline water of these salinities was made as above. (6) The mode of reproduction was observed by growing the ciliates under optimum conditions using egg-custard at 65 ppt salinity and 32°C temperature. (7) Finally, to assess the efficacy of *Fabrea* as live food, it was fed at 10 individual/ml (triplicate) for 10 days larval rearing (20 larvae/beaker) of Japanese flounder (*Paralichthys olivaceus*) from the first day of its mouth opening, in 500 ml of 34 ppt seawater at 18°C. As a control, the rotifer *Brachionus plicatilis* was fed at 10 ind/ml in the same manner to compare larval growth.

The common size range of *Fabrea* (Figure 1) was 120–140 µm accounting for 36.7% of the population. Natural samples showed relatively larger cells of 100–350 µm (Figure 2 *a*). General colour is grey to blackish. Cyst (Figure 2 *b*) diameter was 70–80 µm with a common range of 110–125 µm. Cyst thickness was 3.7–7.4 µm. Body cilia averages 12 µm in length. The width of each adoral zone of membranelle (AZM) is 10 µm. Ordinary food vacuoles measured 2–7 µm. These become enlarged considerably during cannibalism. The macronucleus, noticed as a clear homogenous body, occupies two-thirds the body length in

Table 1. Composition of egg-custard

Ingredients	Quantity
Hen's egg	1
Milk powder	15.0 g
Corn flour (finely ground)	10.0 g
Prawn flour (finely ground)	1.5 g
Agar–agar	2.0 g
Yeast	1.0 g
Vitamin mix	1.0 g
Cod-liver oil	1 ml
Freshwater	60 ml

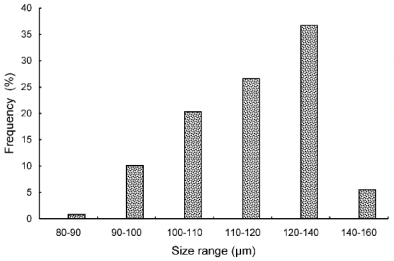

Figure 1. Size distribution of *Fabrea salina*.

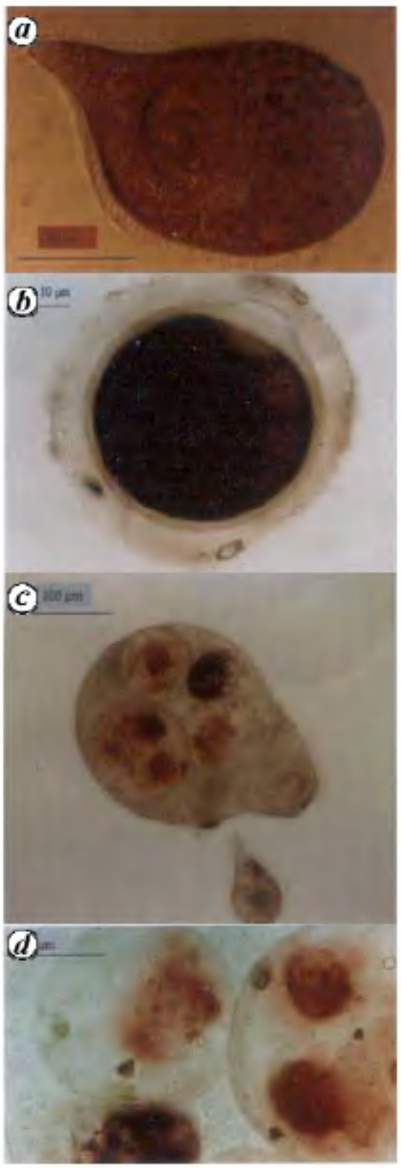

Figure 2. ***a***, *Fabrea salina* (trophic form). ***b***, Cyst of *F. salina*. ***c***, Cannibalistic *F. salina*. ***d***, Intracellular view of cannibalistic *F. salina* showing ingested *Fabrea* cells.

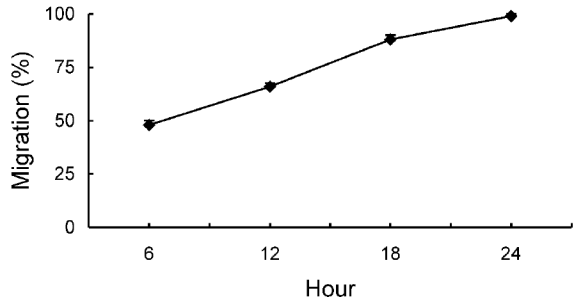

Figure 3. Migration (mean ± SD) of *F. salina* cells to light zone at different hours.

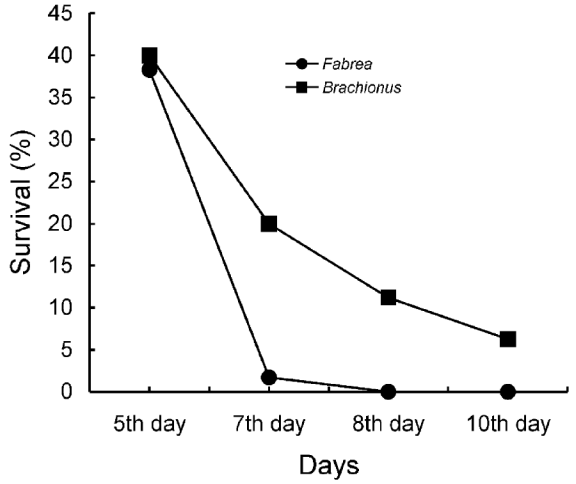

Figure 4. Survival of *P. olivaceus* larvae fed with *Fabrea* and *Brachionus plicatilis*.

stretched state. There is no evidence of existence of contractile vacuole. Generally marine protozoa are devoid of it, as they live in osmotic equilibrium with their environment. Cannibalism, i.e. intraspecific predation has largely been neglected in the dynamics of natural populations. Our experience suggests the food scarcity, higher population density and inadequate culture conditions are conducive factors for cannibalism in *Fabrea*. Starvation resulted in drastic reduction in population density with the appearance of few, slow moving, giant individuals measuring 400–500 μm. An exceptional size of 600 μm has also been recorded. Intracellular observation of cannibalistic *Fabrea* (Figure 2 *c* and *d*) was made possible by placing the cover slip gently over it on the slide so as to just break the plasma membrane. A maximum of nine cells under various stages of disintegration were recorded within the food vacuoles of a single individual. The mega-giant forms were due to increased vacuolization during starvation[7]. They may rely on the stored energy resources, reduced metabolic activity, autophagocytosis, or a combination of these alternatives to survive during starvation[8]. The large forms can be noticed with unaided eyes. We observed cannibalism even during optimum food availability if the population density was higher. We, therefore, suggest partial harvesting be done before population reaches the stationary phase (6–8 days) during culture.

Fabrea shows positive phototaxis (Figure 3). After 6 h exposure, 48% population and after 24 h, almost entire population migrated to transparent half of petri dishes. This behaviour is definitely a good indicator of viability as well as will help easy harvest from the culture systems or natural environment as it orients according to the light stimulus. This ciliate has immense potential to withstand wide salinity variations as no mortality occurred on transferring the cells from 25 ppt to directly 50, 75, 100, 125 or 150 ppt salinities. However, in 175 ppt, the mortality was 25% that rose to 70% in 200 ppt. The mortality was judged by microscopic observation of 1 ml sample on Sadgwick-Rafter counting cell. At first, withdrawing their cilia the ciliates became motionless, adopted an almost spherical shape that followed generally in bursting of the cell. Hatching occurred between 24 and 48 h. Compared to other salinities, the salinity of 40 and 50 ppt resulted in significantly higher hatching percentage, i.e. 14.0 ± 2.0 and 13.3 ± 3.0% respectively (Tukey-Kramer post-hoc test, $p < 0.0001$). At 30, 60, 80 and 100 ppt, the hatching percentage was 7.3 ± 1.5, 7.0 ± 2.0, 2.0 ± 1.0 and 1.0 ± 1.0 respectively.

Beginning of asexual reproduction (binary fission) preceded by cessation of feeding and elongation of the posterior half of the body with a simple constriction in the middle of the cell. The macronucleus became shorter and thicker, elongated into rod-shaped and consequently broke-off into two equal halves. This was followed by deepening of the constriction furrow in the parent cell leading to loss of connection between them and finally separation as two distinct, smaller individuals. The entire process lasted for 3–5 h, of which only 30–35 min were required for detachment after the formation of constriction plane. At optimum growth conditions, the generation period was 16 h. In sexual reproduction (conjugation), the conjugants united along their ventral surfaces of frontal field. Their macronucleus became highly active with twisting and writhing movements followed by their disintegration. The pairing lasted for 40–60 h.

With *Fabrea* as food, the feeding trial showed complete larval mortality on eighth day compared to 11.2% survival on same day with *B. plicatilis* (Figure 4). The larval length (2.96 ± 0.02 mm, $n = 5$ on first day) increased to 3.29 ± 0.04 mm and 3.30 ± 0.11 mm on fifth day with *Fabrea* and rotifers respectively. This rose to 3.44 ± 0.09 mm on tenth day with rotifers as food. Videography for 52 min for initial three days on feeding also revealed non-acceptance of *Fabrea* by larvae. The importance of this ciliate as a potential food source for fish larvae was first mentioned by Morris[9] and later on supported by others[10,11]. At present, this is being used extensively as an experi-

mental animal in biophysical research[12,13].

1. Post, F. J., Borowitzka, L. J., Borowitzka, M. A., Mackay, B. and Moulton, T., *Hydrobiologia*, 1983, **105**, 95–113.
2. Yufera, M., *Investig. Pesq. (Barc)*, 1985, **49**, 493–500.
3. Pandey, B. D. and Yeragi, S. G., *Fish. Chimes*, 1998, **18**, 17–18.
4. Rattan, P., Ansari, Z. A. and Sreepada, R. A., *Trop. Ecol.*, 1994, **35**, 285–294.
5. Pandey, B. D. and Yeragi, S. G., *Aquaculture*, 2004, **232**, 241–254.
6. Pandey, B. D., Yeragi, S. G. and Pal, A. K., *Asian Austral. J. Anim. Sci.*, 2004, **17**, 995–999.
7. Capriulo, G. M. and Degnam, C., *Mar. Biol.*, 1991, **110**, 199–202.
8. Fenchel, T., *Mar. Ecol. Prog. Ser.*, 1982, **9**, 25–33.
9. Morris, R. W., *Bull. Inst. Oceanogr.*, 1956, **1082**, 61.
10. De Winter, F. and Persoone, G., Proceedings of the 10th European Symposium of Marine Biology I, 1975.
11. De Winter, F., Persoone, G. and Benijts-Claus, C., *Fabrea salina*, a promising live food for matriculture purposes. In Proceedings of the 6th Annual Workshop on World Matriculation Society (eds Avault Jr, J. W. and Miller, R.), Seattle, WA, USA, 1976, pp. 429–439.
12. Marangoni, R., Gobbi, L., Vermi, F., Albertini, G. and Colombetti, G., *Acta Protozool.*, 1996, **35**, 177–182.
13. Marangoni, R., Preosti, G. and Colombetti, G., *J. Photochem. Photobiol. B: Biol.*, 2000, **54**, 185–193.

ACKNOWLEDGEMENTS. Dr S. A. H. Abidi, Former Director, Central Institute of Fisheries Education, Mumbai, India is acknowledged for providing facilities to carry out this work. The Ministry of Education, Culture, Sports, Science and Technology of Japan (MONBUKAGAKUSHO) is also acknowledged for the scholarship granted to B.D.P. to carry out a part of the present work.

Received 26 March 2007; accepted 14 December 2007

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Fluoride in water in parts of Raniganj Coalfield, West Bengal

In recent times, there have been media reports[1] that fluoride contamination is widespread in West Bengal. It has been highlighted that 60 blocks in eight districts, viz. Bankura, Barddhaman, Birbhum, Purulia, Midnapur, Malda and West Dinajpur are affected. This had prompted us to study the fluoride content in water from all sources in parts of the Raniganj Coalfield and adjoining Chhotanagpur Gneissic Complex (CGC). The study area has a long history of coal mining with several industrial establishments and traditional agricultural activities.

Fluorine, the 13th abundant element, has a crustal abundance of 625 g/t. In magmatic rocks, fluorine content increases with the increase in silica content. It ranges between 600 and 1000 ppm in granite, 200 and 900 ppm in alkali rocks, 200 and 300 ppm in carbonate rocks and may be as low as 250 ppm in diabase[2]. In the hydrothermal phase, the concentration of fluorine may even exceed 1.0 mg/l (e.g. tourmaline). Concentration of fluorine in sea water is 1.3×10^3 µg/l and is directly proportional to salinity. The residency of fluorine in sea water[3] is 5.2×10^5 yrs.

Fluorine as hydrofluoric acid is also released due to intrusive igneous and volcanic activities in the oceanic and continental domains. In the Raniganj Coalfield (study area) there have been lamprophyre intrusions, which might have contributed fluorine to the pore fluid of the rocks. In the atmosphere, fluorine is found as CFC, HCFC and HFC gases, which when washed down by monsoon precipitation may increase the fluoride content in surface and groundwater. The Younger Toba Ash (YTA) has been deposited over wide areas in the east coast of India following the volcanic eruption of Toba cauldera in northern Sumatra[4]. Precipitation of these ashes and the accompanying rainfall might have also contributed fluorine in the soil and water.

According to the World Health Organization (WHO), the problem of fluorosis is well known from Australia, New Zealand, Kenya, Tanzania, China, Mexico, Chile, Sri Lanka and Bangladesh for some years. India has now joined the group. Fluoride concentration in water up to 1.0 ppm is good for health, but in excess of 2.0 ppm it causes conditions for dense and brittle bone, and dental problems.

Fluorine exists as simply charged F^- ions in minerals like fluorite, or as complex ions in avogadrite (KBF_4) or cryolite (Na_3AlF_6), and also in fluorine-bearing varieties of mica, amphibole. Fluorite occurs as a vein mineral or as a gangue mineral with various metallic ores, especially those of Pb, Zn and Ag. Besides rocks like granite, nepheline syenite, carbonatite or late-stage pegmatite may contain a number of fluorine-bearing minerals, viz. fluorite (CaF_2), fluorapatite [$Ca_3(PO)_2Ca(FCl)_2$], topaz [$Al_2(SiO_4)(OH,F)_2$], tourmaline [$Na(Mg,Fe,Mn,Li,Al)_3Al_6Si_6O_{18}(BO_3)_3(OH,F)_4$], avogadrite ($KBF_4$), cryolite ($Na_3AlF_6$), fluorine-bearing micas (muscovite, biotite, phlogopite) and amphiboles (tremolite, actinolite, hornblende, etc.), and pyrochlore–microlite [$(Na,Ca)_2Nb_2O_6(OH,F)$–$(Na,Ca)_2Ta_2O_6(O,OH,F)$].

The area selected for preliminary appraisal covers two segments in the southwestern and south-central parts of the Raniganj Coalfield, extending onto the Chhotanagpur gneissic terrain (Figure 1) to the south. The western segment (study area I) is bounded by lat. 23°30′N and 23°45′N, and long. 86°47′E and 86°53′E. The eastern segment (study area II) is bounded by lat. 23°30′N and 23°40′N, and long. 87°00′E and 87°15′E. The area covers part of Survey of India toposheet nos 73 I/12 and 73 M/2.

In the study area, Ironstone Shale Formation, Raniganj Formation, and Panchet and Supra Panchet Formations of Gondwana Supergroup were exposed. The Raniganj Formation covers the