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C. R. Biologies 331 (2008) 56–63
COMPTES RENDUS BIOLOGIES
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Ecology / Écologie

Rearing of *Fabrea salina* Henneguy (Ciliophora, Heterotrichida) with three unicellular feeds

Wassim Guermazi [a], Jannet Elloumi [a], Habib Ayadi [a], Abderrahmen Bouain [a], Lotfi Aleya [b,*]

[a] *Unité de recherche 00/UR/0907 « Écobiologie, planctonologie et microbiologie des écosystèmes marins », département des sciences de la Vie, faculté des sciences de Sfax, université de Sfax, route Soukra Km 3,5, BP 802, CP 3018 Sfax, Tunisie*
[b] *Laboratoire de biologie environnementale, UsC INRA 3184, UMR CNRS 6565, université de Franche-Comté, 1, place Leclerc, 25030 Besançon cedex, France*

Received 1 September 2007; accepted after revision 27 October 2007

Presented by Pierre Buser

Abstract

The growth rate of the ciliate *Fabrea salina* was studied in batch cultures in the presence of three feeds, tested separately from each other: the Prymnesiophyceae, *Isochrysis galbana* obtained from pure culture, the Chlorophyceae *Dunaliella salina*, and the commercially available yeast *Saccharomyces cerevisiae. F. salina*, and *D. salina* were harvested below the surface from the first evaporation pond and the crystallizer pond, respectively in multi-pond salterns (Sfax, Tunisia). The highest density of *Fabrea* was recorded with *I. galbana* (26 ind ml^{-1}). However, the greatest length (243 µm) was recorded with *Fabrea* fed with *D. salina*. The lowest density, length and biovolume values were recorded with *Fabrea* fed with *S. cerevisiae*. The ANOVA test showed that density ($F = 18$, d.f. $= 57$), length ($F = 33$, d.f. $= 57$), and biovolume ($F = 19$, d.f. $= 57$) of *Fabrea* fed with yeast were significantly different ($p < 0.001$) from those when *Fabrea* was fed with *D. salina* and *I. galbana*. The ciliate *Fabrea* encountered in the Sfax saltern (Tunisia) might be a valuable food source for Tunisian marine fish hatcheries. ***To cite this article: W. Guermazi et al., C. R. Biologies 331 (2008).***

Résumé

Influence du régime alimentaire sur la croissance en culture d'un cilié extrêmophile *Fabrea salina* Henneguy (1889). L'influence du régime alimentaire sur la croissance en culture d'un cilié extrêmophile, *Fabrea salina*, a été étudiée en culture *batch* en utilisant trois nourritures différentes, constituées par une culture pure de la Prymnésiophycée, *Isochrysis galbana*, de la Chlorophycée *Dunaliella salina* et la levure disponible dans le commerce *Saccharomyces cerevisiae*. *F. salina* et *D. salina* ont été échantillonnées, respectivement, à partir d'un bassin d'évaporation et d'un bassin de cristallisation dans la saline de Sfax (Tunisie). Les plus fortes densités de *Fabrea* sont obtenues avec *I. galbana* (26 ind ml^{-1}). En revanche, les individus de grande taille (243 µm) sont observés dans le cas où *Fabrea* est nourri avec *D. salina*. Les plus faibles valeurs de densité, de taille et de biovolume sont observées avec *Fabrea* nourri de *S. cerevisiae*. Le test ANOVA indique que la densité ($F = 18$, d.d.l. $= 57$) et la taille ($F = 32$, d.d.l. $= 57$) de *Fabrea* nourri avec de la levure diffèrent significativement ($p < 0.001$) de celles de *Fabrea* nourri avec *D. salina* et

* Corresponding author.
E-mail address: lotfi.aleya@univ-fcomte.fr (L. Aleya).

1631-0691/$ – see front matter
doi:10.1016/j.crvi.2007.10.006

I. galbana. *Fabrea* de la saline de Sfax pourrait être utilisé pour des applications aquacoles en raison de sa petite taille n'excédant pas 300 µm et de son court temps de génération. ***Pour citer cet article : W. Guermazi et al., C. R. Biologies 331 (2008).***

Keywords: *Fabrea salina*; Culture; Dynamic; Length; Biovolume

Mots-clés : *Fabrea salina* ; Culture ; Dynamique ; Longueur ; Biovolume

1. Introduction

The heterotrichous ciliate *Fabrea salina* Henneguy (1889) is the dominant protist in hypersaline environments, as it can withstand high salt environments through mechanisms of osmoadaptation and salt tolerance [1,2]. This protozoan has been shown to produce a mucilaginous substance that inhibits the growth of other halotolerant species such as the microalgae *Dunaliella* and different life-cycle stages of the Anostracan *Artemia* [3–5]. These competitive advantages have prompted researchers to investigate the ecophysiology of *Fabrea*, and experimental evidence is now accumulating on the influence of a variety of environmental factors such as nutrient availability, temperature and irradiance level on *Fabrea* dynamics [6–9]. Furthermore, because of the decline in the fish resource, a rapid increase in intensive aquaculture is taking place worldwide, requiring high-quality nutritious feeds. In this respect, pelagic *Fabrea* may be an appropriate candidate to be used by aquafarmers as a feed with minimal cost [10] due to its small length, short generation period, and ability to form resting-cysts. These ciliates have been shown to exhibit remarkable resistance to growth under adverse conditions [11–13] such as those found in multi-ponds salterns. The Sfax solar saltern (Tunisia) has been the focus of a series of hydrobiological studies [14] and we have acquired substantial information on the dynamics of *F. salina* along saline gradients of several ponds [14]. In the present study, the predation by *F. salina* of the Prymnesiophyceae, *Isochrysis galbana*, the Chlorophyceae *Dunaliella salina*, and the yeast *Saccharomyces cerevisiae* was estimated during in vitro incubation by monitoring prey abundances and length spectra. While the flagellate *Isochrysis* is commonly used in aquaculture to enrich zooplankton such as rotifers or *Artemia* [15], the literature on its use as food item for *Fabrea salina* is to our knowledge very scarce. The long-term objective of this study is to consider the large-scale culture of *Fabrea salina* as a food source for the growing aquaculture industry in Tunisia.

2. Materials and methods

F. salina and *D. salina*, were collected using a 5-l Van Dorn bottle below the water surface from multi-pond solar salterns located along the Sfax coast (Tunisia, 34°39′N and 10°42′E) (Fig. 1). *F. salina* was harvested using a plankton net with a 100-µm mesh size, from the first evaporation pond A16 (salinity close to that of seawater: 78.7 ± 8.8 p.s.u.) (Table 1). *D. salina* was collected from the crystallizer pond TS (saturating salt concentrations: 424.5 ± 35.6 p.s.u.), in which the phytoplankton community was entirely composed of this Chlorophyceae (Table 2). The second food item consisted of pure cultures of the Prymnesiophyceae *I. galbana* (Tinamenor S.A. Marisma de Pesués, Cantabria, Spain). Algae and the ciliate were acclimated over three months to continuous illumination (2000 lux) and to Walne's medium (modified from [16], Table 3), respectively.

The commercially available dry yeast, *Saccharomyces cerevisiae*, was also used as a third food item for *Fabrea*. Cultures for experimental purposes were maintained in exponential-growth phase by regular transplants to a fresh medium. The cultures were synchronised, 24 h prior to the beginning of the grazing experiments. This was carried out for 6 days in 200-ml pre-sterilised flasks. Two sets of control (algae without *Fabrea*) and experimental flasks (two replicates each) were used during the experiment in a batch system with a salinity of 50 p.s.u. and maintained at 24 °C in temperature-controlled chambers, and under an illumination of 2000 lux. Ciliates were counted three times in each replicate flask.

The initial concentration of *Fabrea* was 4 cells ml^{-1} and the three food items were inoculated separately from each other, in the morning, during the six days of incubation at a density of 4×10^6 cells ml^{-1}, estimated using a Burcker haemocytometer. The food density was maintained during the experiments by a daily cell enumeration. To stop the experiment, a glutaraldehyde solution (Sigma grade I, final concentration 1%) was added to inhibit protozoan motion [17]. A Sedgwick-Rafter counting cell (Graticules LTD, Ton-

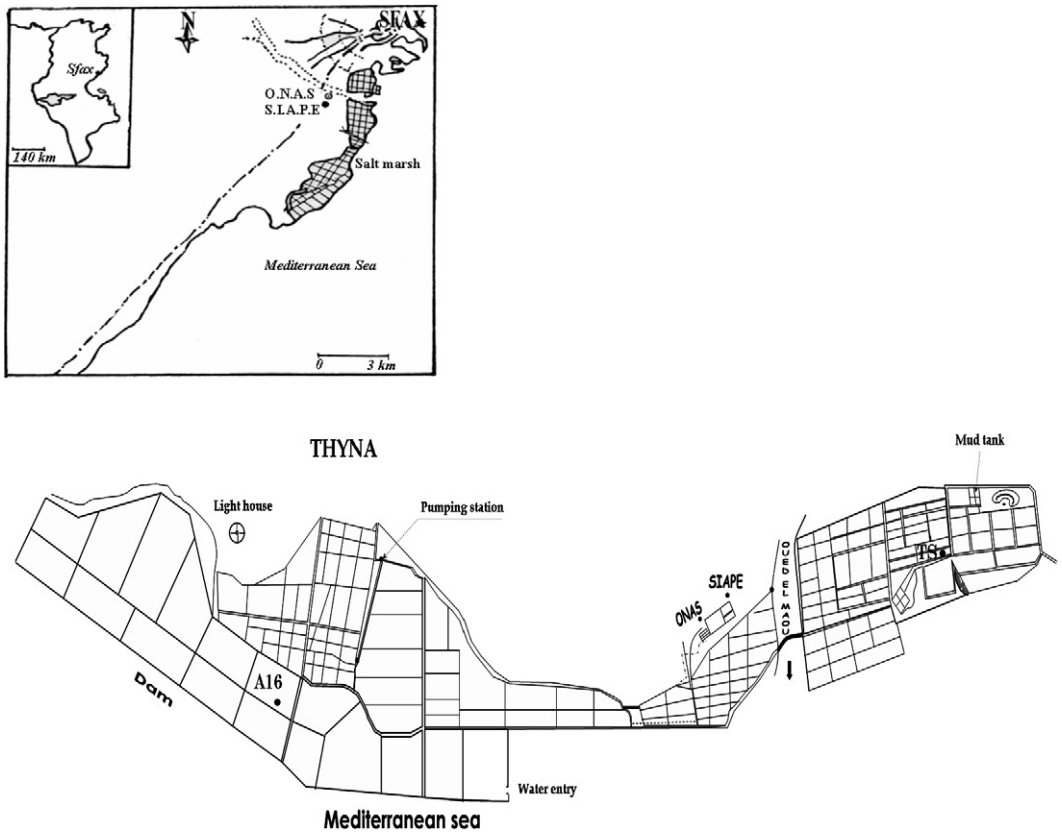

Fig. 1. General map of the geographical location of the multi-pond Sfax salterns along the coast to the south of Sfax (Tunisia) showing the ponds from which were harvested *Fabrea salina* (A16: evaporation pond) and *Dunaliella salina* (TS: crystallizer pond). Modified from [29].

Table 1
Mean values and standard deviation (S.D.) of several physical, chemical, and biological parameters in the saltern

Ponds	A16	TS
Salinity (p.s.u.)	78.7 ± 8.8	424.5 ± 35.6
Temperature (°C)	23.7 ± 7.1	30.6 ± 8.6
pH	7.6 ± 0.7	6.6 ± 0.9
Suspended matter (mg l^{-1})	222.1 ± 148.6	3578 ± 2067
Water density	1.08 ± 0.05	1.27 ± 0.06
Chemical parameters		
Total N (mg l^{-1})	3.7 ± 2.2	10.1 ± 8.7
Total P (mg l^{-1})	1.3 ± 2.4	5.2 ± 3.8
N/P ratio	3.7	1.9
Chlorophyll-*a* (mg M^{-3})	0.097 ± 0.079	0.036 ± 0.01
Biological parameters		
Bacterioplankton ($\times 10^6$ cells ml^{-1})	7.7 ± 5.8	25.3 ± 14.7
Phototrophic picoplankton ($\times 10^5$ cells ml^{-1})	4.1 ± 2.7	0.0
Nanoplankton ($\times 10^5$ cells ml^{-1})	18.6 ± 8.3	0.8 ± 0.4
Phytoplankton ($\times 10^6$ cells ml^{-1})	0.4 ± 0.3	1.2 ± 1.4
Ciliates ($\times 10^4$ cells ml^{-1})	4.7 ± 3.5	0.0
Zooplankton ($\times 10^4$ ind m^{-3})	0.2 ± 0.2	0.0

bridge, Kent, UK), mounted on a Type Leica DM LS2 microscope (20X magnification), was used to estimate the average daily changes in *Fabrea* numbers; *Fabrea* length and width were estimated using a micrometer and the biovolume of *Fabrea* was measured according to [18].

Table 2
Specific composition of phytoplankton, ciliates and zooplankton sampled in ponds A16 and TS of the Sfax saltern

Ponds	A16	TS
Phytoplankton		
Diatoms	*Navicula* sp.	
	Pinnularia sp.	
	Nitzschia sp.	
	Surirella sp.	
	Gyrosigma sp.	
Dinoflagellates	*Oxyrrhis marina*	
	Prorocentrum sp.	
	Gymnodinum sp.	
	Protoperidinum sp.	
Chlorophyceae		*Dunaliella salina*
Ciliates	*Urotricha* sp.	
	Fabrea salina	
	Euplotes sp.	
Zooplankton		
Copepods	*Acartia grani*	
	Acartia clausi	
	Harpacticus littoralis	
	Bryocamptus sp.	
	Tisbe longicornis	
	Mesochra sp.	
	Micosetella sp.	
	Copepodits	
	Nauplii	
Rotifers	*Brachionus urceolaris*	
	Brachionus calyciflorus	
Other zooplankton		
	(Mainly larvae)	

The specific growth rate was calculated using the following formula:

$$\mu(\text{day}^{-1}) = 1/t \times \ln(A_t - A_0)$$

with t being the incubation time (days), A_0 and A_t the culture density, respectively at the beginning and at the end of the experiment.

2.1. Statistics

Mean and standard deviation (SD), as well as boxplots are reported when appropriate. Simple linear regression was used when analyzing how each food item could explain the relation between the length and biovolume of *Fabrea*. One-way ANOVA followed by a post-hoc comparison using Tukey's test [19] was applied to identify significant differences between food treatments for (*i*) density, (*ii*) length, and (*iii*) biovolume of *Fabrea*.

Table 3
Chemical composition of the medium used to culture *Fabrea salina*. MT: Metric tons

Elements	Concentrations (g MT^{-1})
Chlorides	16560
Sodium	9210
Sulphates	2324
Calcium	350
Potassium	343
Bicarbonates	127
Brominates	19
Strontium	7
Boron	5
Fluorine	1.2
Manganese	1.359
Molybdenum	0.690
Lithium	0.170
Rubidium	0.110
Iodine	0.070
Aluminium	0.062
Zinc	0.035
Copper	0.0036

3. Results

3.1. Fabrea *density and growth rate*

The density of *Fabrea* fed with *D. salina* did not exhibit significant changes from the start until the third day of the experiment. The growth rate was low ($\mu = 0.12\ \text{day}^{-1}$) and cell numbers did not exceed 2.5 (± 1.41) cells ml^{-1} (Fig. 2a). However, from day 4 until the end of incubation, *Fabrea* cell numbers increased rapidly ($\mu = 0.94\ \text{day}^{-1}$), reaching 17 ± 2.12 cells ml^{-1} on the 6th day (Fig. 2a). When cultured with *I. galbana*, the density of *Fabrea* increased until day 3 ($\mu = 0.50\ \text{day}^{-1}$, 18.25 ± 1.77 ind ml^{-1}) and then collapsed (0.5 ± 0.71 cells ml^{-1}). From the 4th day until the end of the experiment, *Fabrea* grew strongly ($\mu = 1.31\ \text{day}^{-1}$) up to 25.75 ± 6.01 cells ml^{-1} (Fig. 2b). A similar pattern in the distribution of *Fabrea* was recorded when the protozoan was fed with the yeast *S. cerevisia*. However, no latency time on the fourth day was observed (Fig. 2c). Indeed, *Fabrea* grew ($\mu = 0.18\ \text{day}^{-1}$) until the second day ($7 \pm 0.71$ cells ml^{-1}), then its density decreased until the fourth day (1.75 ± 1.77 cells ml^{-1}). From day 4 onwards, the ciliate growth rate increased again ($\mu = 0.31\ \text{day}^{-1}$), yielding a cell density of 4.5 ± 2.83 cells ml^{-1}. From the first day until the end of each grazing experiment, the specific growth rates (μ) of *Fabrea* fed with *Isochrysis*, *Dunaliella* and *Saccharomyces* were 0.3, 0.24 and 0.02, respectively (Fig. 3).

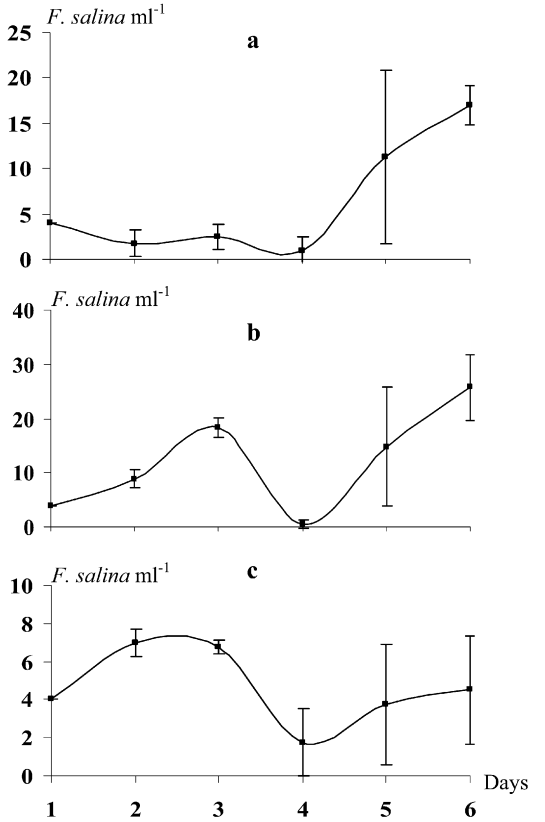

Fig. 2. Daily evolution of the density of *Fabrea salina* reared in the presence of *Dunaliella salina* (**a**), *Isochrysis galbana* (**b**) and *Saccharomyces cerevisiae* (**c**). The vertical bars represent the standard deviation.

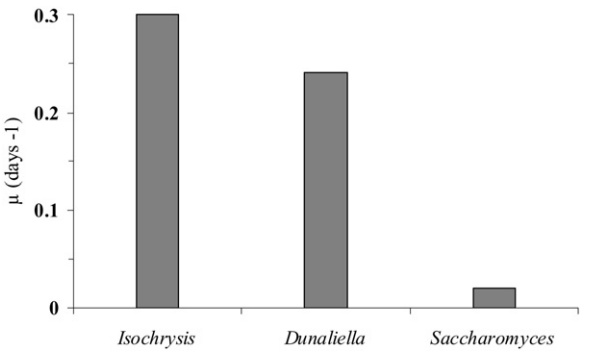

Fig. 3. Specific growth rates (μ) of *Fabrea* fed with different preys from the start to the end of grazing experiments.

3.2. *Length and biovolume of* Fabrea

When fed with *D. salina*, the length of *Fabrea* varied from 200 ± 9 μm, recorded in the latency phase to 281.5 ± 15 μm, recorded on the second day, corresponding to a biovolume of 15.8×10^5 μm (Table 4). The average length of *Fabrea* was 243.2 ± 31.71 μm, cor-

Table 4
Mean and standard deviation (SD) of length (μm) and biovolume ($\times 10^5$ μm^3) of *Fabrea salina* reared separately from each other with different preys

Feeds	*Dunaliella salina*						*Isochrysis galbana*						*Saccharomyces cerevisiae*					
Parameters	Length			Biovolume			Length			Biovolume			Length			Biovolume		
Days	Media	Min	Max	Media	Min	Max	Media	Min	Max	Media	Min	Max	Media	Min	Max	Media	Min	Max
2	281.5	140.5	290.2	15.8	7.9	16.3	165.0	148.1	207.4	4.2	2.7	6.0	140.0	110.6	160.6	4.5	2.9	7.2
3	251.9	180.0	270.1	14.2	6.4	15.2	191.0	163.0	222.2	7.5	5.1	11.7	152.0	118.5	177.8	4.7	2.8	7.4
4	200.0	163.0	266.7	19.2	6.7	30.0	251.9	180.1	265.0	2.6	6.4	14.6	148.1	100.5	180.3	4.3	3.1	7.9
5	260.7	190.0	278.3	14.7	8.7	15.7	219.0	89.0	104.0	10.7	7.4	13.3	177.8	120.4	210.0	7.4	4.7	8.9
6	234.6	192.6	266.0	20.0	7.9	39.0	180.0	133.0	222.0	11.8	5.5	28.8	165.0	148.0	207.0	5.6	4.3	8.6

responding to a biovolume of $18.6 \pm 10.15 \times 10^5$ µm^3. During the exponential growth phase, the length of *Fabrea* was high (260.7 ± 46 µm), with a biovolume of $14.7 \pm 9.91 \times 10^5$ µm^3 (Table 4).

When *I. galbana* was used as diet, the length of *Fabrea* varied from 165 ± 20.95 µm on the second day ($4.23 \pm 3.07 \times 10^5$ µm^3) to 251.9 ± 10 µm recorded on the fourth day ($2.6 \pm 1.02 \times 10^5$ µm^3). Mean *Fabrea* biovolume recorded over the 6 days of experiments (mean ± SD = $8.15 \pm 5.14 \times 10^5$ µm^3), corresponded to a mean length of 189.01 ± 30.83 µm. However, at the end of the experiment, the length of *Fabrea* decreased, to reach 180 ± 40.15 µm.

When fed with dry yeast, the length of *Fabrea* ranged between 140 ± 20 µm and 177.8 ± 5 µm (mean ± SD = 160.49 ± 22.63 µm), corresponding to a mean biovolume of $5.33 \pm 1.81 \times 10^5$ µm^3. Conversely to the temporal distribution of the prey-predator couples *Fabrea–Isochrysis* and *Fabrea–Dunaliella*, the average length of *Fabrea* cultured with yeast increased throughout the experiment (Figs. 4 and 5).

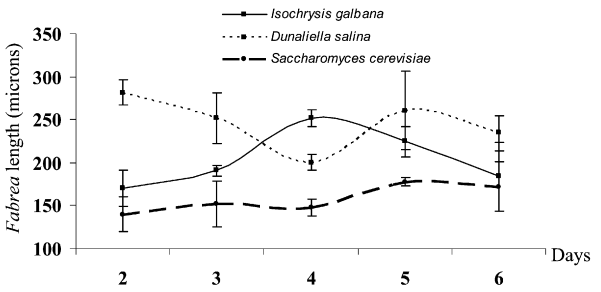

Fig. 4. Daily evolution of the length of *Fabrea salina* in the presence of *Dunaliella salina*, *Isochrysis galbana* and *Saccharomyces cerevisiae*. Vertical bars represent the standard deviation.

4. Discussion

The results indicate that under controlled experimental conditions, *F. salina* was able to grow when its diet consisted of *D. salina*, *I. galbana* and *S. cerevisiae*. Indeed, continuous light, a temperature of 24 °C, a salinity of 50 p.s.u. and a small water volume (200 ml) were sufficient to yield optimal growth of *Fabrea*. This is consistent with the findings of several authors, who showed that *Fabrea* can develop with various nutritional items [13]. Moreover, Rattan et al. [20] indicated that *Fabrea* can even grow with fermented wheat and rice grains. However, Repak [6] indicated that this ciliate does not grow in the presence of the Cyanobacteria *Synechococcus* spp. Many other protists have been successfully cultured, such as *Favella* sp., *Uronema* sp., *Gymnodinium* sp. [21,22]. A high growth rate ($r = 0.60$ day^{-1}) was also recorded when the Tintinnid *Favella* sp. was fed with the Prymnesiophyceae *Prymnesium parvum* [22]. One should also bear in mind that it is of fundamental importance to keep growth and ingestion constant to evaluate growth yield in ciliates feeding different preys [23–25]. Although yield is constant, absolute biomass production may be higher in one prey than in another. Both high density and growth rates of *F. salina* were recorded when the diet consisted of *I. galbana* (26 cells ml^{-1}, $r = 1.31$ day^{-1}). Indeed, the

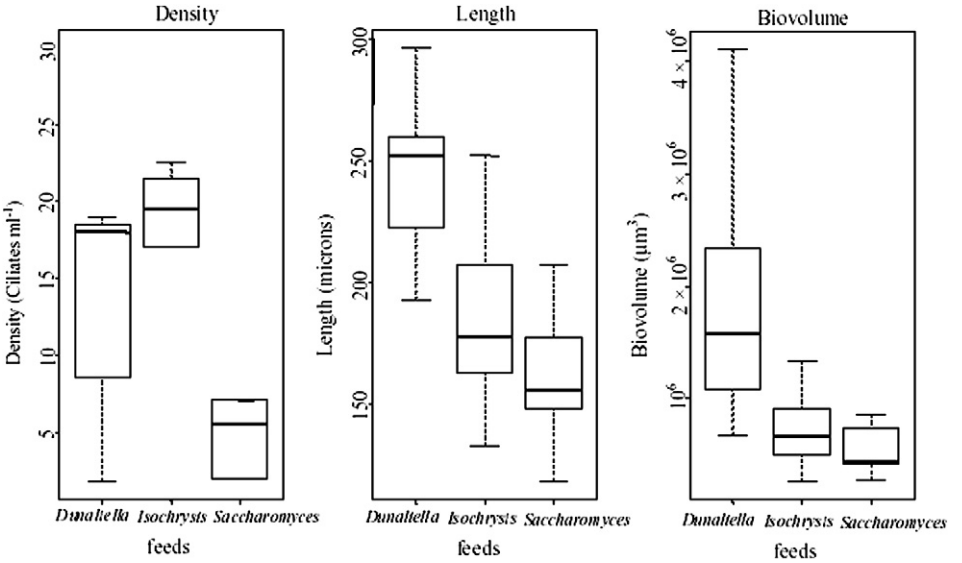

Fig. 5. Boxplot showing the distributions of the variables: density, length, and biovolume of *Fabrea* for the three treatments with *Dunaliella*, *Isochrysis* and *Saccharomyces*. (The thick line in the middle of the box indicates the median value.)

peak density recorded on the third day indicated a short-generation period of *Fabrea*, which may suggest that *I. galbana* had a high nutritional value, as already reported by Brown [26]. To our knowledge, the culture of *Fabrea* with *Isochrysis* has never been reported, so we were unable to compare our findings with those of others. The only available data concerned the genera *Strobilidium* and *Strombidium*, which exhibit a maximum growth rate of 2.2 day^{-1} when fed with *Isochrysis* [27]. Although the effects of this flagellate on *Fabrea* development were remarkable, both the length and biovolume of the ciliate were smaller than those recorded when the *Fabrea* diet consisted of *D. salina*. This discrepancy may suggest that *Fabrea* underwent reproduction, as evidenced by the existence of two peaks of *Fabrea* density recorded on the third and sixth days (Fig. 2b), coinciding with a decrease in cell dimensions on the sixth day (Fig. 4). In support of this, we also found the weakest correlations between the length and biovolume of *Fabrea* fed with *I. galbana* ($r = 0.45$, d.f. $= 27$, $p < 0.01$), versus *D. salina* ($r = 0.77$, d.f. $= 17$, $p < 0.01$) and the yeast ($r = 0.95$, d.f. $= 10$, $p < 0.01$). The highest densities of *Fabrea* were found when the latter was fed with *D. salina* and *I. galbana*. Pandey and Yeragi [13], who worked under similar experimental conditions, reported similar results with *D. salina*, but they did not consider *I. galbana* as a food item. They recorded cell densities of 44 and 64 *Fabrea* ml^{-1} when grown in 1 and 5-l containers, respectively. Concerning the yeast *S. cerevisiae*, our results indicate that it was not a good food item for *Fabrea*, because the ciliate induced the lowest growth rate, cell length, and biovolume. The ANOVA analysis shows that both the density ($F = 18$, d.f. $= 57$), length ($F = 33$, d.f. $= 57$) and biovolume ($F = 19$, d.f. $= 57$) of *Fabrea* fed yeast differed significantly ($p < 0.001$) from those of *Fabrea* fed both with *D. salina* and *I. galbana* (Tables 5 and 6). This difference may be explained by the deterioration of the medium throughout the experiment induced by yeast, as already suggested by [20]. Indeed, the high *Fabrea* density recorded on the second day implies a very rapid growth generation period which was strongly stimulated by *S. cerevisiae* at the beginning of the experiments. Yeast is widely used as a dietary supplement to support the growth of several species such as rotiferans and the anostracan *Artemia*. Pandey and Yeragi [13] found a density of 20 *Fabrea* ml^{-1} when fed with yeast at concentrations of 5 $mg\,l^{-1}$. Overall, our results are slightly lower than those reported by Repak [6,7] and Pandey and Yeragi [13]. The maximal length of *Fabrea* recorded in this study (207 μm) is similar to that recorded by Dolapsakis et al. [28], who cultured *F. salina* obtained from a Greek saltern in which the salinity varied from 60 to 144 p.s.u. Furthermore, both the length and biovolume of *Fabrea* recorded in this study were higher than those found in the Sfax saltern. The values reported by [14] from this ecosystem did not exceed 111 μm and 180.1×10^3 μm^3, for salinities ranging between 70 and 170 p.s.u., respectively.

Table 5
Results of ANOVA analysis to assess the effect of different food combinations on the density, length and biovolume of *Fabrea*

Parameters	*F* value	d.f.	*P* value
Density	18	57	6.38×10^{-7}***
Length	33	57	3.48×10^{-10}***
Biovolume	19	57	5.61×10^{-7}***

Significant level *** at $p < 0.001$.

Table 6
Post-hoc comparison using Turkey's test to identify significant differences among treatments

Feeds	Density	Length	Biovolume
Isochrysis–Dunaliella	0.12	0.00*	0.00*
Saccharomyces–Dunaliella	0.00*	0.00*	0.00*
Saccharomyces–Isochrysis	0.00*	0.02*	0.45

* p values below 0.05 indicate significant differences between the two treatments.

In conclusion, our study indicates that the ciliate *Fabrea salina* was able to grow with different feeds, and may be a good candidate species as food source for the Tunisian aquaculture industry. To improve our overall understanding of the various preys–*Fabrea* relationships, we are currently investigating the ecophysiological responses of *Fabrea* to changes in light, temperature, salinity, together with its fatty acid composition.

Acknowledgements

We gratefully acknowledge support from the staff of the Sfax Saltern Campany. We would like to thank especially Dr. Roberto Marangoni for helpful advices and comments on the manuscript. The pure culture of *Isochrysis galbana* was kindly provided by Dr H. Chavanne (Lazzaro Spallanzani Institute, Milan, Italy). This work was conducted as part of a collaborative project between the University of Sfax (Tunisia) and the University of Franche-Comté (Besançon, France). We thank the Tunisian Ministry of Research and Technology for financial support.

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