

Estuarine nursery under environmental stress with emphasis on the benthonic microalgae community.

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ABSTRACT: Estuarine nursery under environmental stress with emphasis on the benthonic microalgae community. In the Favela do Caranguejo a poor community on the Capibaribe River estuary is a fish farming nursery where biological data were collected for identifying and quantifying benthonic algae, biomass, phaeophytin, and primary production. Biomass represented by chlorophyll "a", showed slower daily growth in the rainy season than in the dry season, when two biweekly cycles of fast intense biomass accumulation were recorded. There were intense pulses in the first successive stages, in both periods (dry and rainy), typical of an environment under intense physical and chemical variations, causing communities frequent stress.

Key-words: Benthic algae, eutrophication, environmental stress.

RESUMO: Viveiro estuarino sob estresse ambiental com ênfase na comunidade microalgal bentônica. Na Favela do Caranguejo, localizada às margens do estuário do Rio Capibaribe, encontra-se um viveiro de piscicultura onde foi realizada a presente pesquisa. Para este trabalho, foram coletados dados biológicos como identificação e quantificação das algas bentônicas, biomassa, feofitina e produção primária. A biomassa expressa através da clorofila "a", teve um crescimento diário mais lento na estação chuvosa que na estação seca, quando dois ciclos quinzenais de rápida e intensa acumulação de biomassa foram encontrados. Intensos pulsos na produção primária nos primeiros estádios da sucessão foram registrados, em ambos os períodos (seco e chuvoso), o que caracteriza um ambiente sujeito a intensas variações físico-químicas, submetendo as comunidades biológicas a freqüente estresse.

Palavras-chave: Algas Bentônicas, eutrofização, estresse ambiental.

Introduction

Most industrial projects and urban expansion are almost always accomplished without any prior study of their environmental impact, even less on measures to contain or to minimize their impact on the environment.

An example of this environmental degradation is the state of the River Capibaribe estuary (Pernambuco, Northeastern, Brazil), which, in spite of its social, economical, and cultural importance and for dividing the city of Recife, it receives considerable amounts of industrial and domestic effluent from both the urban zone and several sites up river.

Communities attached to submerged surfaces are of fundamental importance in understanding the metabolism of aquatic ecosystems, as periphytic algae and/or benthonic microalgae can represent up to 80 to 90% of the total primary productivity in shallow water (Wetzel, 1990). The attached community accumulates large quantities

of pollutants, such as insecticides, herbicides, radioactive elements, heavy metals, organic pollutants (Wetzel, 1983 and 1990; Stevenson et al.,1996)

The benthonic community is controlled by environmental conditions with variations in composition and species diversity, and physiological changes like abiotic factors (Chamixaes, 1991). Less attention has been paid to the effects that multiple disturbances have on ecosystems and organisms although many suffer the impacts of these multiple disturbances and other stressors (Paine et al., 1998).

This study is important because there is a growing interest in the role of the microbenthonic community as a primary producer in aquatic ecosystems and its ecological role affecting the distribution of plants and animals; it is also an excellent bio-indicator of water quality and provides food and shelter for several organisms.

Study area

The fish nursery farm where this work was performed is in the Favela do Caranguejo in the Afogados neighbourhood of Recife, Pernambuco. It is on the edge of the Capibaribe river's tidal estuary between 08°04' and 08°05'S and 34°54' and 34°55'W (Fig. 1). The estuarine nursery is a semi-confined water mass occupying an area of approximately 1.52 hectares with a volume of 3,560m³ and an average depth of 1.0m, where fish such as Mugilidae (e.g.Tainha, Curimã, Carapeba, Sauna, etc) are cultivated.

According to Silvestre (1997), the annual characteristics of the water in the nursery range from: 25.0 to 29.0°C (temperature); pH 7.4 - 8.9; 8,160 to >20,000mS.cm⁻¹ (conductivity); >10.0mgO₂.L⁻¹ (biochemical oxygen demand); not detected to 19.0mgN.L⁻¹ (total nitrogen); 0.2 to 0.5mgP.L⁻¹ (total dissolved phosphorus); 0.12 - 0.23mgAl.L⁻¹ (aluminium); <0.0003 to 0.04mgPb.L⁻¹ (lead); 0.03 to 0.05mgZn.L⁻¹ (zinc); 0.02 to 0.03mgMn.L⁻¹ (manganese); 0.01 to 0.03mgCr.L⁻¹ (chromium), and 0.22 to 0.28mgFe.L⁻¹ (iron).

Average air temperature in the study area was 24.1°C and 26.2°C in the rainy and dry seasons, respectively. The precipitation reached an average of 11.1mm/day in the rainy season, and 2.9mm/day in the dry season.



Figure 1: Partial aerial view of Recife, showing Capibaribe river (1) located at Favela do Caranguejo; the river arm (2) and the fish farming nursery (3). Scale: 1:10,000. (Source: FIDEM).

Capibaribe River crosses several urban nuclei in the downstream stretches near the sea; several industrial plants are present in the watershed and, in the metropolitan area of Recife, and the river receives domestic sewage. All the residues of industrial pollution are discharged into the river, most of them are stored in sediments. Since the nursery is closely associated with the river, it is submitted to all pollutants entering the water.

Material and methods

Field collection

Microscope glass slides were used for colonization and observation of the succession occurring in the benthos, and to determine the primary production and biomass in the same space and time.

Glass slides were placed in polystyrene (PET) barred boxes to protect them from grazing by large predators like shrimps, crabs, and fishes. These boxes were anchored to the bottom of the fish nursery by nylon lines. Samples were taken after 02, 04, 07, 09, 11, 14, 16, 18, 23, 30, 37, and 44 days (T2, T4, T7, T9, T11, T14, T16, T18, T23, T30, T37, and T44, respectively). At each harvest, eight colonized slides were selected and water was sampled for physical and chemical analysis. This experimental procedure was made in the rainy (June and July, 1997) and dry (January and February, 1998) seasons.

Benthonic Microalgae Analysis

Benthonic microalgae were collected from the Favela do Caranguejo fish nursery, fixed with formol to 2.0% neutralized with borax, and identified by microscope at 100x.

For the quantitative analysis, 2.0ml of acetic lugol solution was added to samples. Benthonic algae counting was performed in an inverted microscope by the Utermöhl (1958) method modified by Chamixaes (1991).

Organism quantification was by the count method for random fields, recommended for populations with clumped distribution (Uhelinger, 1964; Bicudo, 1990).

The minimum number of counting fields was determined by the graphic method of curve stabilization obtained by starting from added species with increase of shown area (Boudouresque, 1971), 60 fields were deemed adequate.

The benthonic algae biomass is the chlorophyll "a" quantity in the samples, determined by the Lorenzen (1967), monochromatic method modified for substratum area by Chamixaes (1991). The primary benthonic algae production was measured by the dissolved oxygen evolution method (Gaarder & Gran, 1927), modified by Chamixaes (1991). Total organic production (TOP) was estimated by the $\text{mgO}_2 \times 0.375 = \text{mgC}$ (Vollenweider, 1969). Assimilation rate (AR) of the fish nursery benthonic algal community was calculated by the Vollenweider et al. (1969) method modified by Chamixaes (1991). The following equation was used:

$$\text{AR} = \frac{\text{mgC. cm}^{-2} \cdot \text{h}^{-1}}{\text{mgChla. cm}^{-2}} \quad (\text{in mgC.mgChla}^{-1} \cdot \text{h}^{-1})$$

Results

Figure 2 shows the variations in benthonic microalgal community growth in the Favela do Caranguejo fish farm nursery. There was very intense growth in the first 15

days of the dry season, followed by an abrupt fall in density, probably due to substratum saturation. In the rainy season, there was less growth over the whole experimental period.

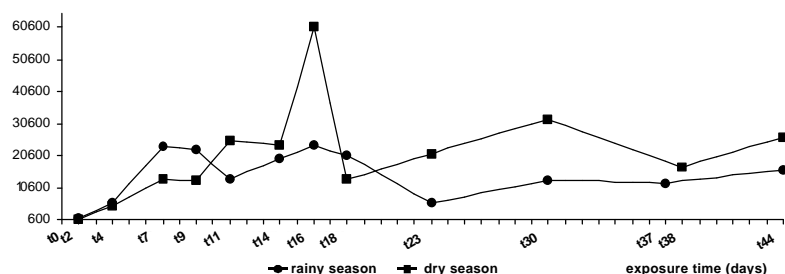


Figure 2: Benthonic microalgae density (cells.cm⁻²) during succession in the rainy and dry season at Favela do Caranguejo fish nursery, Recife, Pernambuco, Brazil.

In the rainy season, the benthonic algal biomass growth curve was of sigmoid type, with the exponential growth phase from the beginning of colonization to the 9th exhibition day; this was followed a stationary phase until approximately the 30th day (Fig. 3). However in the dry season, the microalgal community presents a “J” type growth curve, with the biomass exponentially increasing up to the 18th day, falling soon after, growing again and then falling at the end of experiment (Fig. 3).

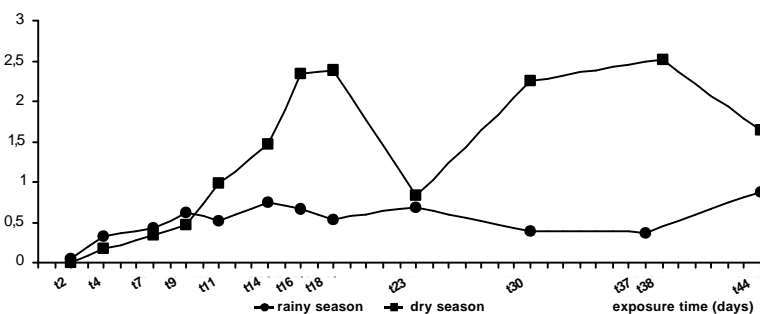


Figure 3: Biomass in terms of chlorophyll “a” (mg.cm⁻²) in the benthonic microalgae community during succession, in the rainy and dry season at Favela do Caranguejo fish nursery, Recife, Pernambuco, Brazil.

During the rainy season, chlorophyll “a” ranged from 0.05mg.cm⁻² on T2, to 0.88mg.cm⁻² on T44 (Table I). During the dry season, no chlorophyll was detected at T2, but biomass was 2.52mg.cm⁻² at T38 (Table II). Phaeophytin varied from 0.02mg.cm⁻² at T2 to 3.88mg.cm⁻² at T44 in the rainy season; it was not detected at T2, and attained 3.88mg.cm⁻² at T16 in dry season (Tables I and II); phaeophytin increased during both seasons.

Gross primary productivity (GPP) in the fish nursery varied in the rainy season; it dropped from 0.0179mgO₂.cm⁻² on T2 to 0.0055mgO₂.cm⁻² on T37 (Table I). In the dry season, it was 0.0077mgO₂.cm⁻² on T14, and 0.0210mgO₂.cm⁻² on T16 (Table II). GPP began with an intense fluctuation in both seasons, but after the 23rd day of the dry season no significant change was observed.

Net primary productivity (NPP), during the rainy season ranged from 0.0009mgO₂.cm⁻², on T7 to 0.0136mgO₂.cm⁻² on T23; in the dry season, it dropped from 0.0161mgO₂.cm⁻² on T16 to 0.0005mgO₂.cm⁻² on T44 (Tables I and II). Total organic production (TOP) abrupt dropped from 0.8790mgC.h⁻¹.cm⁻² on T23 to 0.1662mgC.h⁻¹.cm⁻²

on T37 in the rainy season, and in the dry season, it dropped from 0.7430mgC.h⁻¹.cm⁻² on T2 to 0.2327mgC.h⁻¹.cm⁻² on T14 (Tables I and II). During the rainy season, TOP was at its highest on T23 and lowest on T37; this could indicate that the community reached a peak condition and then began to decline. There is a similarity between TOP and biomass growth curve in the rainy season, when TOP grew up to the 23rd day and then fell, and biomass grew until the 30th day and then decreased. Such relationships were not seen during the dry season (Table II). Breathing activity (BA) varied through the rainy season dropping from 0.0227mgO₂.cm⁻² on T14 to 0.0015mgO₂.cm⁻² on T44; and in the dry season, it varied from 0.0033mgO₂.cm⁻² on T7 to 0.0138mgO₂.cm⁻² on T9 (Tables I and II). Assimilation rate (AR) of microbiota benthonic algal ranged from 10.8200mgC.mgChla⁻¹.h⁻¹ on T2 to 0.3264mgC.mgChla⁻¹.h⁻¹ on T14 in the rainy season; in the dry season, it was not detected, in the 2nd day and was up to 3.2681mgC.mgChla⁻¹.h⁻¹ on T4.

Table I: Biomass in terms of chlorophyll "a" and phaeophytin, gross (GPP) and net (NPP) primary productivity, total organic production (TOP), and breathing activity (BA) of benthonic microalgae at Favela do Caranguejo fish nursery during the rainy season.

Sample time (day)	Chlorophyll a (mg/cm ²)	Phaeophytin (mg/cm ²)	GPP (mgO ₂ .cm ⁻²)	NPP (mgO ₂ .cm ⁻²)	TOP (mgC.h ⁻¹ .cm ²)	BA (mgO ₂ .cm ⁻²)
T2	0.05	0.02	0.0179	0.0133	0.5410	0.0046
T4	0.32	0.08	0.0158	0.0131	0.4776	0.0026
T7	0.42	0.27	0.0073	0.0009	0.2206	0.0065
T9	0.62	1.09	0.0087	-	0.2630	0.0104
T11	0.51	0.95	0.0158	0.0031	0.4776	0.0128
T14	0.75	1.47	0.0081	-	0.2448	0.0227
T16	0.67	1.73	0.0137	0.055	0.4141	0.0082
T18	0.54	1.31	0.0119	0.0056	0.3600	0.0063
T23	0.69	0.67	0.0291	0.0136	0.8790	0.0155
T30	0.39	1.49	-	-	-	0.0170
T37	0.37	1.49	0.0055	-	0.1662	0.0111
T44	0.88	3.61	0.0135	0.0120	0.4080	0.0015

Table II: Biomass in terms of chlorophyll "a" and phaeophytin, gross (GPP) and net (NPP) primary productivity, total organic production (TOP), and breathing activity (BA) of benthonic microalgae at Favela do Caranguejo fish nursery during the dry season.

Sample time (day)	Chlorophyll a (mg/cm ²)	Phaeophytin (mg/cm ²)	GPP (mgO ₂ /cm ²)	NPP (mgO ₂ /cm ²)	TOP (mgC.h ⁻¹ .cm ²)	BA (mgO ₂ /cm ²)
T2	ND	ND	0.0082	0.0016	0.7430	0.0066
T4	0.16	0.00045	0.0173	0.0132	0.5229	0.0040
T7	0.33	0.36	0.0129	0.0095	0.3899	0.0033
T9	0.47	0.98	0.0126	0.0031	0.3808	0.0138
T11	0.97	0.55	0.0176	0.0079	0.5320	0.0097
T14	1.46	0.33	0.0077	0.0034	0.2327	0.0042
T16	2.33	3.88	0.0210	0.0161	0.6347	0.0049
T18	2.38	1.47	0.0126	0.0025	0.3808	0.0100
T23	0.85	1.53	0.0185	0.0082	0.5592	0.0104
T30	2.25	1.08	0.0128	0.0065	0.3869	0.0063
T38	2.52	1.15	0.0151	0.0028	0.4564	0.0123
T44	1.63	1.71	0.0102	0.0005	0.3083	0.0097

GPP= gross primary productivity;
 NPP= Net primary productivity;
 TOP= total organic production;
 BA= breathing activity

Three classes of microalgae were found in the Favela do Caranguejo fish nursery: Cyanophyceae, Chlorophyceae, and Bacillariophyceae (Figures 4 and 5). During the rainy season, Diatoms (Bacillariophyceae) were dominant throughout the succession, always corresponding to more than 65% of algal populations in the samples, and representing more than 90% on T2, T11, T16, T18 and T30 (Figure 6). At this time of the year, the main diatom found in the benthonic microalgal community was *Navicula agnita*. Green algae (Chlorophyceae) had some representatives until T21, notably the *Closterium sp* species. Cyanophyceae was better represented, starting from T23, the main species being *Lyngbya sp*.

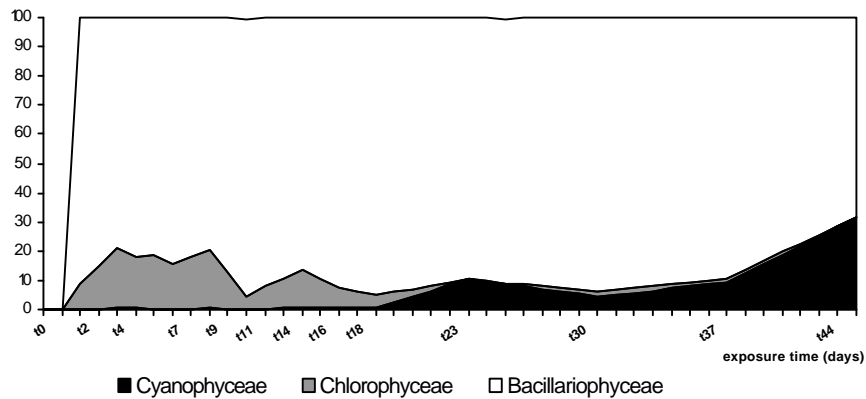


Figure 4: Algae Relative density (%) during succession, in the rainy season at Favela do Caranguejo fish nursery, Recife, Pernambuco, Brazil.

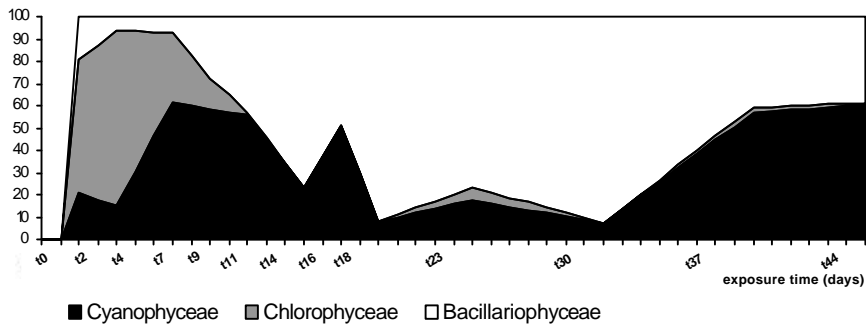


Figure 5: Algae Relative density (%) during succession in the dry season at Favela do Caranguejo fish nursery, Recife, Pernambuco, Brazil.

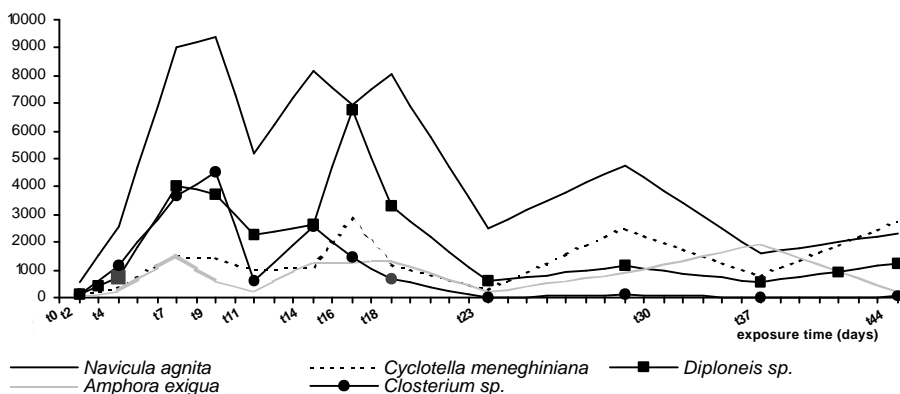


Figure 6: Main microalgae species density (cells.cm⁻²) during succession, in the rainy season at Favela do Caranguejo fish nursery, Recife, Pernambuco, Brazil

However in the dry season, the first step was dominated by Chlorophyceae, mainly *Closterium* sp representing almost 80% of the benthonic microalgal population on T4 (Fig. 7). After T7 there was alternating dominance between Cyanophyceae and Bacillariophyceae, but the Cyanophyceae, even when dominate, never represented more than 65% of the microalgae. From these, the main species, in terms of density were *Lyngbya* sp and *Spirulina* sp but Bacillariophyceae represented over 90% of the benthonic microbiota algal in the nursery on T18 and T30. The diatom species with higher frequency in the dry season were *Amphora exigua*, *Cyclotella meneghiniana* and *Navicula agnita*.

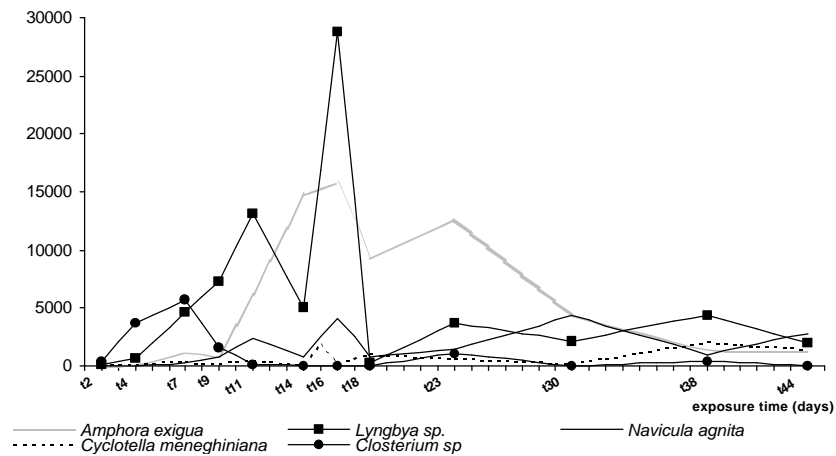


Figure 7: Main microalgae species density (cells.cm⁻²) during succession in the dry season at Favela do Caranguejo fish nursery, Recife, Pernambuco, Brazil.

Discussion

The metabolic processes of the benthonic microalgae community in the Favela do Caranguejo fish farm nursery are very quick. This was due to two factors: the high contribution of nutrients in the nursery, and the fact that the benthonic community was in direct contact with the sediment, where there is larger nutrient deposition, also seen by the high benthonic microalgal assimilation rate. Autotrophic cells grow more rapidly in the presence of ammonium than nitrate. Thompson et al. (2002) and Li et al. (1990) noted that, in presence of ammonium growth rate is double that of controls. Eutrophication can be accelerated if the main form of exported nitrogen is ammonium and can be reduced in the water bodies by effective phosphate uptake (McClelland & Valiela 1998). The input of sewage in this nursery contributes to ammonium load, and since there was low phosphate uptake, eutrophication is accelerated.

The degradation processes are also intense, since the phaeophytin levels soon overtook the chlorophyll, starting from the 9th day in the rainy season, and from the 7th in the dry season.

Stress is well identified when examining community structure and species succession than when expressed through standing-stock in chlorophyll "a" or cell density (Chamixaes, 1991). The role of physiological stress in structuring communities may be greater in warmer climates (Bertness et al., 1999).

In an experiment to create a mature benthonic microalgal community, Thompson et al. (2002) found maximum chlorophyll "a" (11.39mg.cm⁻²) after 14 days exposure. In the macrocosm experiment with the benthonic microalgal community of Favela do

Caranguejo fish farm nursery, the maximum Chlorophyll "a" was detected in rainy season after 14 days (0.75 mg/cm²) and after 16 days (2.33mg/cm²) in dry season. In field and laboratory experiments, the time to obtain a mature biofilm is approximately the same as observed by many authors (Silvestre 1994; Gould & Gallagher,1990) for lentic ecosystems.

Thompson et al. (2002) observed an increase in Chlorophyll "a" concentration even in the tanks with mature biofilm after 28 days of substrate exposition. The increase of Chlorophyll "a" after establishing a mature biofilm in the first life cycle (0 to 28 days), represents a secondary succession process in this nursery and was recorded after 44 days of experiment in both seasons.

There was no net primary productivity on some days in the rainy season; this can be an indication of high heterotrophic activity, consuming all the oxygen surplus produced by the autotrophic organisms of the nursery benthonic biota. It can still indicate anoxia periods in the sedimentary layer, even during the day. In polluted estuaries oxygen concentrations fall and can become anaerobic under extreme conditions (Perkins, 1974), causing intense fish mortality. Extreme changes in environmental factors like salinity, temperature, and nutrients can induce low growth and performance of algae (Erick & Azanca, 2002).

In the rainy season, assimilation rate was higher than the dry season because the experiment was performed in a shallow ecosystem (less than 1m depth) and the precipitation, in spite of dilution, promotes displacement of the water mass and causes lixiviation on the edges. Also the presence of stronger winds can cause a more nutrient to be liberated, released from sediment into the water column and thus improving nutritional conditions for autotrophic organisms. This fish farm nursery is a eutrophic system in all the physical, chemical, and biological characteristics and therefore has low diversity.

The presence of the benthonic algae species in this nursery denotes a typical estuarine flora resistant to domestic and industrial pollution, and thus are excellent bio-indicators (Moreira Filho & Valente-Moreira 1981; Branco 1986), to characterize an estuary under threat from humans (Table III).

Table III: Species found in the Favela do Caranguejo fish farm nursery and their ecological meaning.

Species	1	2
<i>Amphora angusta</i>	Sea, coast, epiphyte, euryhaline	-
<i>A. exigua</i>	Sea, coast, euryhaline	-
<i>Cocconeis dirupta</i>	Sea, coast, epiphyte	-
<i>Coscinodiscus excentricus</i>	Sea, coast, polyhaline, euryhaline, tytoplanktonic	-
<i>C. radiatus</i>	Sea, planktonic, neritic	-
<i>Cyclotella meneghiniana</i>	Limnetic, coast, oligohaline, halophyta.	-
<i>Fragilaria pinnata</i>	Limnetic, coast, oligohaloby, oligosaprobry	-
<i>Melosira moniliformis</i>	Salobra, sea, coast	-
<i>Navicula cryptocephala</i>	Limnetic, alkaliophyta, oligosaprobry	Resistant to industrial pollution from paper factory residues, phenol, etc.
<i>Closterium</i> sp	-	This genera are particularly resistant to spillings rich in chrome
<i>Lyngbya</i> sp	-	This genera is characteristic of polluted waters and can produce cyanotoxines
<i>Pediastrum tetras</i>	-	Grow well in waters polluted by paper industrial spillings which are toxic to most algae

Sources: 1-Moreira Filho & Valente-Moreira (1981) and 2-Branco (1986).

The biofilm was dominated by diatoms that occurring in the first 2 days of the experiment (Thompson et al., 2002). In the rainy season (Silvestre, 1997), there were slower growth rates with diatom dominance, and in the dry season, growth rates were faster with green algae dominance.

Contrary to expectation, biofilm removal can increase the risk of pathogenic bacteria development in tanks with biofilm exporting less phosphorus (Thompson et al., 2002).

Considering organism seasonality, there are poor methods for combating epiphytes (pest weed and epiphytic algae), herbivory, and diseases (Ask 1999). As emphasized in this paper the ecophysiology of the epiphytic community can help elucidate seasonal growth patterns to combat stress and disease on biological cultures (e.g. fish).

Conclusions

From the results in this study of benthonic microalgae succession at the Favela do Caranguejo fish farm nursery, Recife, Pernambuco, Brazil, it was concluded that:

a) The benthonic microalgae biomass growth curve at Favela do Caranguejo nursery was of the sigmoid type in the rainy season, and "J" type in dry season, indicating higher growth rates in the dry season; b) The high phaeophytin concentrations before halfway in the substrata exhibition time indicate early senescence of the benthonic algal community; c) The community metabolism of the Favela do Caranguejo nursery was accelerated due to factors such as high temperature, high pH, high nutrient readiness, and high assimilation rate; d) Diatoms (Bacillariophyceae) were dominant during the rainy season and blue-green algae (Cyanophyceae) during the dry season; e) This ecosystem is an eutrophic environment, and environmental stress causes community reduction in terms of biomass, production, and species diversity when compared to other similar ecosystems.

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