Characterization of the Zooplankton Community of the Secondary Wastewater Treatment System of an Oil Refinery in Southern Brazil

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RESUMO

A comunidade zooplanctônica da Bacia de Aeração do Sistema de Tratamento Secundário de Efluentes da Refinaria Alberto Pasqualini – REFAP, PETROBRAS, RS foi analisada no presente trabalho enfatizando a composição e abundância dos organismos. Amostras foram coletadas quinzenalmente entre Janeiro e Julho de 1996 em três pontos no sistema: entrada, meio e saída. Protistas, rotíferos e copépodos foram observados e enumerados no laboratório. Ao todo, 17 espécies de protistas, 10 espécies de rotíferos e uma espécie de copépodo estavam presentes na comunidade durante o período do estudo. A maioria dos taxa estava presente em todos os pontos de coleta com exceção de um gênero de protista e três gêneros de rotíferos. Os protistas foram dominantes nos três pontos de amostragem, seguidos por rotíferos e copépodos. Entre os protistas, *Vorticella* sp., *Paramecium* sp., *Coleps* sp. e *Euglena* sp. foram os mais abundantes durante o periodo de estudo, chegando a uma densidade máxima de mais de 3.000 células/L. *Brachionus* spp., *Phylodina* sp., *Trichocerca* sp., *Keratella* sp. e *Notomata* sp. foram os rotíferos mais abundantes no sistema de tratamento alcançando densidades médias de mais de 250 indivíduos/L. Náuplios de *Metacyclops mendocinus* (Wierzejski, 1892) estiveram presentes am todos os pontos de coleta, alcançando uma densidade média de 26 indivíduos/L. Análises de correlação não apontaram interações significativas entre variáveis ambientais e abundância de organismos zooplanctônicos, sugerindo que relações mais complexas talvez estejam presentes no sistema.
Palavras-chave: *Brachionus*, copépodos, *Metacyclops mendocinus*, protistas, rotíferos

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ABSTRACT

In the present work, the zooplankton community of a secondary treatment system of an oil refinery was analyzed emphasizing its composition and abundance of organisms. Samples were collected twice a month between January and July 1996 from three stations in the system: entrance, middle, and outlet. Protists, rotifers, and copepods were observed and enumerated in the laboratory. A total of 17 species of protists, 10 species of rotifers, and one species of copepod composed the zooplankton community during the study period. The majority of taxa was present in all three station with exception of one protist genus, and three genera of rotifers. Protists were dominant in the three sampling stations followed by rotifers and copepods. Among protists, *Vorticella* sp. (Linnaeus, 1767), *Paramecium* sp. Ehremberg, 1838, *Coleps* sp. Nitzsch, 1827, and *Euglena* sp. Ehremberg, 1838, were the most abundant taxa during the study period, reaching a maximum mean density of more than 3.000 cells/L. *Brachionus* spp. Pallas, 1776, *Phylodina* sp. Ehremberg, 1830, *Trichocerca* sp. Lamarck, 1801, *Keratella* sp. Bori de St. Vincent, 1822, and *Notomata* sp. Ehremberg, 1830 were the most abundant rotifers in the treatment system, with mean densities that reached more than 250 inds/L. Nauplii of *Metacyclops mendocinus* (Wierzejski, 1892) were present at all stations reaching a maximum mean abundance of 26 inds/L. Correlation analyses failed to show statistically significant interactions between environmental variables and zooplankton abundance, suggesting that more complex relationships may be governing the system.

Key words: *Brachionus*, copepods, *Metacyclops mendocinus*, protists, rotifers

INTRODUCTION

The structure of zooplankton communities has been the subject of many studies in freshwaters of temperate (ALLAN, 1976; LONSDALE; COULL, 1977; BROWNLEE; JACOBS, 1987) and tropical regions (ODEBRECHT, 1988; LIMA et al., 1996; ESPÍNDOLA et al., 2000; NOGUEIRA, 2001), but only few researches have focused on the composition of the zooplankton in polluted environments (CAIRNS et al., 1972; GOODNIGHT, 1978; BARBIERI; ORLANDI, 1989; PARK; MARSHALL, 2000; SHUKLA; GUPTA, 2001). Members of the zooplankton community are widely utilized as bioindicators of environmental pollution because they are easy to collect, to identify, and, as a whole community, they respond quickly to environmental stresses (GANNON; STEMBERGER, 1978; HELLAWELL, 1986). The structure of the zooplankton community is especially influenced by climatic, physical and chemical parameters, biogeographical factors, and biotic interactions, therefore some species could be found in a wide range of environmental conditions, while others are limited by many physical and chemical factors including pollution (GANNON; STEMBERGER, 1978; DUMONT, 1999; NEVES et al., 2003).

Among the zooplankton community, rotifer abundance appears to be linked to trophic conditions of the environment. For example, JOSÉ DE PAGGI (1976) reported that rotifers represented 99.4% of the zooplankton community in an eutrophic water body. MATSUMURA-TUNDISI and co-workers (1990) observed a high abundance of rotifers in relation to cladocerans and copepods in a highly polluted environment. Calanoid copepods are generally abundant in oligotrophic environments, while cyclopoids and cladocerans dominate in eutrophic waters (MARGALEF, 1983; WETZEL, 2001). Investigations on the structure of the zooplanktonic community and abundance of organisms coupled with analyses of chemical and physical parameters of the water, are important to obtain basic knowledge on the species diversity of a given water body as well as its underlying dynamics (NEVES et al., 2003).

The majority of studies about the composition of zooplankton communities has focused on rotifers, cladocerans, and copepods, without including the protozooplankton (BEAVER; CRISMAN, 1982). Although protists are not included in routine evaluations of the zooplankton due to difficulties in identification (PACE; ORCUTT, 1982), they play an important role in the grazing of bacteria and energy transfer to higher trophic levels (BEAVER; CRISMAN, 1982; AZAM et al., 1983). In wastewater treatment systems, protists, especially ciliates, are important in the production of an effluent with low turbidity and high dissolved oxygen content, due to predation pressure on bacteria populations, but only few studies have included these organisms in the analyses of zooplankton communities (CURDS, 1965; CURDS et al., 1968; CURDS, 1973; SMALL, 1973; ESTEBAN et al., 1991; CURDS, 1996).

The goals of the present study are to characterize the structure of the zooplankton community in a wastewater secondary treatment system, and to investigate spatial fluctuations in the community, with emphasis on the protozooplankton and its relationships with environmental variables.

MATERIALS AND METHODS

Study Site: Alberto Pasqualini Oil Refinery is located in Canoas City, Rio Grande do Sul State, Southern Brazil. An average of 16.000 mm^3 of oil is refined on a daily basis, and, as a result of this activity, a considerable amount of pollutants such as oils, phenols, sulphide, suspended solids, and heavy metals are produced. Before being deposited in the Sapucaia Creek, the resultant byproducts go through a primary treatment system, where the water is separated from the oil, and a secondary treatment system constituted by an aerated lagoon. This lagoon has approximately 31.000 m^2 in area (88.6m in width, 36.4m in length. and 1.90m in depth), 19 aerators (4 underwater and 15 on the surface) and a residence time of about 10 days. In addition to the industrial wastewater, the lagoon processes domestic sewage from the neighborhood. The bacteria introduced into the system through the raw sewage process the pollutants and contribute to the reduction of the wastewater toxicity (HORAN, 1991). After completing the residence time, the wastewater is damped in the Sapucaia Creek which runs to the Sinos River.

Zooplankton Community: The zooplankton community was sampled twice a month between January and July 1996. Whole water samples were collected below the surface with a bucket with a final volume of 2 liters from three stations in the system: entrance, middle, and outlet. A 150 mL sub sample from each station, was fixed with formaldehyde 4% to observe rotifers and copepods. Another 150 mL from each station was fixed with Mercurium Chloride and Bromophenol Blue as suggested by PACE and ORCUTT (1981) to observe protists. The rest of the sample was distributed in bottles to perform physical and chemical analyses and to observe organisms “in vivo” in the laboratory. Protists and rotifers were counted using a Sedgewick-Rafter counting chamber and tentatively identified to genus or species level based on key morphological features characterized by DONNER (1966), KUDO (1971), RUTNER-KOLISCO (1974), KOSTE (1978), CORLISS (1979), and LEVINE et al. (1980). Whole chambers were counted to a total of 300 individuals of a given species or five ml of sample, whichever came first. For copepod abundance estimation, 40 mL sub-sample was taken from each sample with a graduated pipette and analyzed using a dissecting microscope. Copepods were identified to species level based on keys published by SENDACZ and KUBO (1982) and REID (1985).

Physical and Chemical variables: Abiotic parameters such as water temperature, pH, dissolved oxygen, conductivity, alkalinity, hardness, and chloride content were measured in the laboratory. Water temperature (C) was measured with a thermometer, pH by an Analion TM 600 pHmeter, dissolved oxygen by an Analion OX901 oxymeter, and conductivity by an Analion C701 conductivimeter. Alkalinity was estimated by reaction with HCl 0.01N (APHA, 1985), hardness was measured by reaction with EDTA 0.01M (APHA, 1985), and chloride content by reaction with $AgNO_3$ (APHA, 1985). For chlorophyll *a* analyses, water samples from each station were kept in the dark, under refrigeration until extraction was performed. Samples were run through a 0.45 μm filter, and chlorophyll *a* was extracted with acetone 90%. Samples were read in a spectrophotometer (PMK2, Zeiss Inc.) at wavelengths of 663 and 750 nm (WETZEL; LIKENS, 1991).

Correlation analyses were performed to evaluate fluctuations in the zooplankton community and environmental variables. These analyses were run using Sigma Stat Version 2.0 (SPSS Inc.).

RESULTS

Table 1 shows mean values of physical and chemical variables recorded during the study period for each sampling station. Water temperature was very similar in the three sampling points during the study period. The lowest temperature was observed in July and the highest in January. Values of pH were similar for the three stations and were close to neutrality. Dissolved oxygen content varied considerably when the three stations were compared. The lowest value (0.3 mg/L) was recorded for station 1. Stations 2 and 3 presented similar values of dissolved oxygen during the study period. Values of electric conductivity, alkalinity, and chloride contents were very similar for the three stations for the study period. Values of chlorophyll *a* were higher in the samples collected in May, for the three stations. For all other sampling times, measurements were very similar for all stations.

The zooplankton community in the secondary treatment system of the Alberto Pasqualini oil refinery was composed by 17 species of protists, 10 species of rotifers and 1 species of copepod (Table 2). All protists, except the genus *Chlamydomonas* Ehremberg, 1833, were found in all three sampling stations (Table 2). *Chlamydomonas* sp. was found only in the entrance of the system (Station 1). Among the rotifers, *Brachionus* sp., *Keratella* sp., and *Trichocerca* sp. were not present in all stations (Table 2). *Brachionus* sp. was recorded in the entrance (Station 1) and outlet (Station 3) of the system, *Trichocerca* sp. was found only in the middle station (Station 2), while *Keratella* sp. was observed at stations 2 and 3. The only species of copepod present in the system, *Metacyclops mendocinus* (Wierzejski, 1892) was found at all three stations (Table 2). All life stages (nauplii, copepodites, and adults) were present during the study.

Greatest taxon richness was observed in February for station 1, with a total of 18 species, in January for station 2 with a total of 21 species, and in March for station 3 with 20 species total. The lowest number of taxa was recorded in July for all stations when 4, 6, and 7 species were observed in Stations 1, 2, and 3 respectively.

Regarding relative abundance, protists were dominant in the three sampling stations, followed by rotifers and copepods, which showed lowest abundance in station 2 (Figure 1). Among protists, when absolute abundance is considered, *Vorticella* sp., *Coleps* sp., and *Paramecium* sp. were the dominant species at station 1 during the study period (Figure 2). At stations 2 and 3, *Vorticella* sp. and *Coleps* sp. were also abundant, and *Euglena* sp. was the flagellate dominant at station 2 with a mean density higher than 200 cells/L (Figure 2). Four rotifer species were abundant at station 1 during the study period: *Brachionus, angularis* (Gosse, 1851); *Brachionus calyciflorus* (Pallas, 1766), *Phylodina* sp., and *Trichocerca pusila* (Lauterborn, 1898), as can be seen in Figure 3. Similarly to station 1, at station 2 *B. calyciflorus*, *B. angularis*, and *Phylodina* sp. were most abundant, with peaks of mean abundance that varied between 140 and 16 ind/L. At station 3, *B. angularis* and *B. calyciflorus* were also dominant with peaks of 232 ind/L and 35 ind/L respectively. *Notomata* sp. and *Phylodina* sp. were also abundant, but the mean density was much lower in comparison than that found for *B. angularis* (13 ind/L and 35 ind/L respectively). The only copepod species present in the study site was the Cyclopoid *Metacyclops mendocinus*. Since no species comparison was possible, estimates of abundance for nauplii and adults were performed. Nauplii of *M. mendocinus* were dominant at all stations during the study period (Figure 4). At station 1 they reached a maximum mean density of 26 ind/L while adults presented a maximum mean density of 2 ind/L. Density of nauplii was much lower at station 2 in comparison with station 1. A mean density of 5 ind/L was recorded for that station. On the other hand, the highest density reached by adults was the same observed for station 1 (1 ind/L). At station 3, nauplii reached a maximum mean density of 19 ind/L while adults had a mean density of only 0.7 ind/L.

Correlation analyses failed to show any statistically significant correlation between species composition and environmental variables suggesting that the fluctuations in the zooplankton community may be governed by more complex interactions.

DISCUSSION

The system analyzed in this study is shallow, presenting small fluctuations in water level, pH values close to neutrality, and water temperature very similar among sampling stations. In wastewater treatment systems, fluctuations of pH could affect the toxicity of certain compounds resulting in a higher toxicity of the effluent as a whole (CÔTÉ, 1976). Dissolved oxygen concentration varied considerably during the study period, despite the low water depth observed in the system. The lowest values of dissolved oxygen were recorded for the entrance of the aerated lagoon, which could be linked to the high organic input of raw sewage originated in the refinery. The intense bacterial activity in this station could have contributed to the decrease of dissolved oxygen observed during the study period.

The electric conductivity could be used to measure pollution because this variable at polluted sites is always higher than in non-polluted areas (COLE, 1975; WETZEL, 2001, KLEEREKOPER, 1990). Chloride contents in natural environments are highly variable, and their concentration depends on edaphic, atmospheric, and pollutant factors (COLE, 1975). In oil refineries, chlorides could be originated from the processing of petroleum, while in domestic sewage, human and animal excretions contribute to the high levels of this ion. In the present study, inputs of raw sewage in the system probably contributed to the high, but uniform, values of conductivity and chloride contents observed for all sampling stations.

Chlorophyll *a* content is a measurement frequently used to estimate phytoplankton biomass and to predict eutrophication of freshwater environments (WETZEL, 2001; DODDS, 2002). Effluents, in general, present a low concentration of chlorophyll *a* due to their high turbidity, low light penetration, and low photosynthetic activity (BOZELLI, 1991). The effluent evaluated in the present study presented a concentration of chlorophyll *a* that varied between 0.267 and 10.54 mg/m^3, lower than reported for natural polluted environments, and following the observation made by BOZELLI (1991).

The zooplankton community in the secondary treatment system of the Alberto Pasqualini Oil refinery was dominated by protists during the study period. Rotifers were also abundant, and were represented by a total of 10 species, and copepods were represented by a single species (*Metacyclops mendocinus*). The structure of the community was characteristic of a polluted environment, since this kind of habitat generally presents high abundance of protists, especially ciliates (PACE; ORCUTT, 1981; BEAVER; CRISMAN, 1989; 1990). However, detailed studies focusing species composition and the role of these organisms in the planktonic community in natural or artificial polluted environments are lacking, especially in the Southern Hemisphere .

According to HORAN (1991) the grazing pressure of ciliates on bacteria in wastewater treatment systems is responsible for a reduction in the concentration of bacteria and, as a consequence, a production of an effluent of good quality. CURDS and co-workers (1968) demonstrated experimentally that effluents containing ciliates presented a significantly lower turbidity when compared to effluents with no ciliates. They attributed this difference to the grazing pressure exerted by ciliates on bacteria. Although protists play an important role in wastewater treatment systems, they are frequently neglected from surveys in these environments due to difficulties in identification and preservation of species.

Few studies including protozooplankton analyses in wastewater treatment systems have pointed out that the environment is dominated by small, generally bacterivorous ciliates and flagellates. HORAN (1991) observed that protists in treatment systems, especially ciliates, present a high richness of species, but a low number of individuals of each species. In the present study, the protist community was dominated by ciliates of the genera *Vorticella* and *Paramecium*, and by a photosynthetic flagellate of the genus *Chlamydomonas*. Both genera of ciliates are predominantly bacterivorous with high filtration rates. STOESSEL (1989) observed that high densities of *Vorticella* spp. were related to high abundance of bacteria. At the same time, peritrichs are highly sensitive to changes in oxygen concentration, and it has been demonstrated that *Vorticella* spp. were unable to survive in an oxygen concentration below 2 mg/L (BICK, 1972). In the study area, oxygen concentrations were always higher than 2 mg/L, which made the environment suitable for growth and development of *Vorticella* sp.

Rotifers are commonly found in wastewater treatment systems where the effluent is biologically treated and aerated. Although their occurrence in this kind of environment is well known, there are only few records of rotifers in treatment systems (SLÁDECEK, 1983), especially in the Southern Hemisphere. In natural environments, high abundance of rotifers is generally related to eutrophic conditions, with only few species recorded as bioindicators of oligotrophic environments (GANNON; STEMBERGER, 1978). In the present study 10 rotifer species were recorded, all of which are known for natural environments. The genus *Brachiounus* was the most abundant in the aerated lagoon and was represented by three species. According to SLÁDECEK (1983), the species of *Brachionus* found in this study show a wide range of tolerance to pollutants and are abundant in eutrophic environments.

Similar to ciliates, rotifers, in general, feed on bacteria and small algae and are capable of filtering one thousand times their own body volume in one hour (BRÖNMARK; HANSSON, 1998). This high grazing pressure of rotifers on bacteria may contribute for the production of a good quality effluent as suggested by some authors for ciliates (CURDS et al., 1968; HORAN, 1991; CURDS, 1996). In addition to the availability of food, the numerical abundance of rotifers in eutrophic or non-eutrophic environments could be due to the fact that they are r-strategists organisms, with a short life-cycle, and a wide tolerance to fluctuations of environmental factors (WETZEL, 2001; NEVES et al., 2003).

Copepods are organisms broadly represented in aquatic ecosystems and constitute an important portion of the biomass in the zooplankton community of freshwater and marine environments (MARGALEF, 1983; WETZEL, 2001). Among copepods, the Order Calanoida is more representative in oligotrophic environments, while the Order Cyclopoida is abundant in eutrophic systems. The only species of copepod found in the present work was the cyclopoid *Metacyclops mendocinus*, that represented only a small part of the zooplankton community during the study period. This species was reported as abundant in natural (MONTÚ; GLOEDEN, 1986) and artificial eutrophic environments (SENDACZ, 1984), but not in secondary treatment systems. According to SENDACZ and co-workers (1985), *M. mendocinus* is a characteristic species of eutrophic environments and is able to survive in habitats with low oxygen concentrations.

In populations of copepods, the high abundance of juvenile forms was pointed out by several authors (PAGGI; JOSÉ DE PAGGI, 1990; VASQUEZ; REY, 1992; SAMPAIO; LOPEZ, 2000; NEVES et al., 2003), and was also found in the present study. Factors that could influence the abundance of young forms include predation by invertebrates and availability of food (WETZEL, 2001). The presence of juveniles is of great importance for the structure of zooplankton communities since different young forms and adult stages occupy different trophic niches (NEVES et al., 2003). The availability of food would also contribute for the differential abundance of juveniles and adults, since naupliar, copepodite, and adult stages feed on different particle sizes. In the present study, the high abundance of naupliar stages may be explained by availability of food, since nauplii feed on algae, bacteria, and detritus, abundant in the system.

CONCLUSIONS

Protists, rotifers and copepods were present in the zooplankton community of the secondary treatment system of Alberto Pasqualini Oil Refinery during the whole study period, and were represented by species characteristic of polluted environments. Since species fluctuations could not be explained by correlations between environmental variables and abundance of other taxa, they may be related to food availability, competition, predation, and/or seasonal fluctuations.

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Table 1: Mean values of physical and chemical variables recorded between January and July 1996 for the entrance (Station 1), middle (Station 2), and outlet (Station 3) of the Secondary Treatment System of Alberto Pasqualini Oil Refinery.

Variable	Station 1	Station 2	Station 3
Temperature (^oC)	22.6 (± 3.6)	22.4 (± 3.7)	22.5 (± 3.7)
pH	7.2 (± 0.4)	7.4 (± 0.3)	7.5 (± 0.4)
Conductivity (µs/cm)	1350.5 (± 610.7)	1220.5 (± 440.1)	1230.4 (± 430.9)
Oxygen	4.5 (± 2.5)	6.2 (± 1.7)	6.4 (± 1.6)
Alkalinity (mg/L $CaCO_3$)	70.4 (± 22.5)	61.1 (± 18.2)	61.2 (± 18.3)
Hardness (mg/L $CaCO_3$)	229.2 (± 178)	186.2 (± 161.4)	189.2 (± 172.7)
Chloride Contents (mg/L Cl^-)	468.5 (± 310.4)	398.2 (± 301.8)	382.9 (± 269.6)
Chlorophyll *a* (mg/m^3)	1.8 (± 2.2)	2.8 (± 3.8)	2.2 (± 2.7)

Table 2: Composition of the zooplankton community in the entrance (Station 1), middle (Station 2) and outlet (Station 3) of the Secondary Treatment System of the Alberto Pasqualini Oil Refinery between January and July 1996.

Species	Station 1	Station 2	Station 3
Protists			
Euglena sp.	X	X	X
Peranema sp.	X	X	X
Phacus sp.	X	X	X
Chlamydomonas sp.	X		
Actinophrys sp.	X	X	X
Askenasia sp.	X	X	X
Trachelius sp.	X	X	X
Coleps sp.	X	X	X
Lacrymaria sp.	X	X	X
Dileptus sp.	X	X	X
Didinium sp.	X	X	X
Paramecium sp.	X	X	X
Vorticella sp.	X	X	X
Halteria sp.	X	X	X
Aspidisca sp.	X	X	X
Euplotes sp.	X	X	X
Stylonichia sp.	X	X	X
Rotifers			
Phylodina sp.	X	X	X
Notommata sp.	X	X	X
Trichocerca pusilla	X	X	
Trichocerca sp.		X	
Brachionus angularis	X	X	X
Brachionus calyciflorus	X	X	X
Brachionus budapestinensis	X	X	X
Brachionus sp.	X		X
Keratella sp.	X	X	X
Lecane bulla	X	X	X
Copepods			
Metacyclops mendocinus	X	X	X

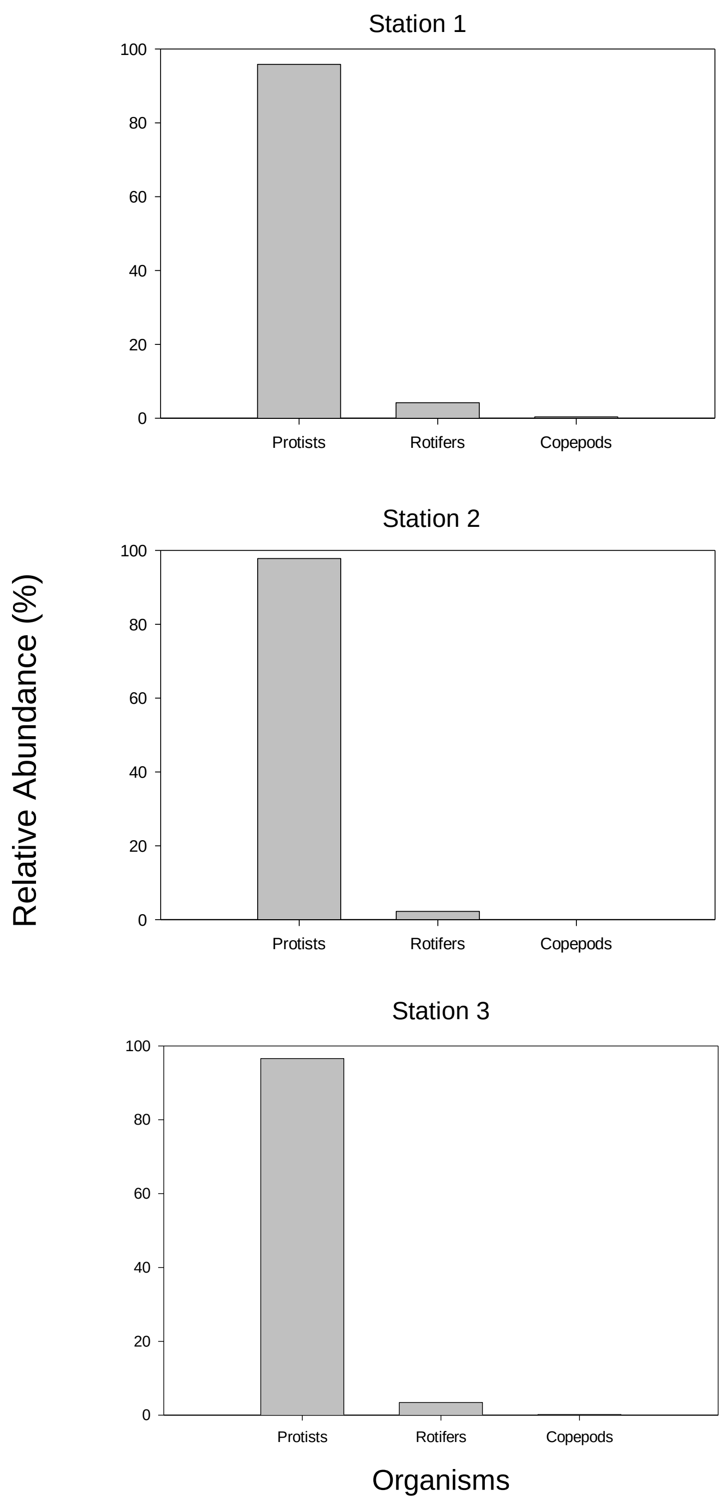

Figure 1: Relative abundance (%) of protists, rotifers, and copepods in the secondary treatment system of the Alberto Pasqualini Oil Refinery between January and July 1996.

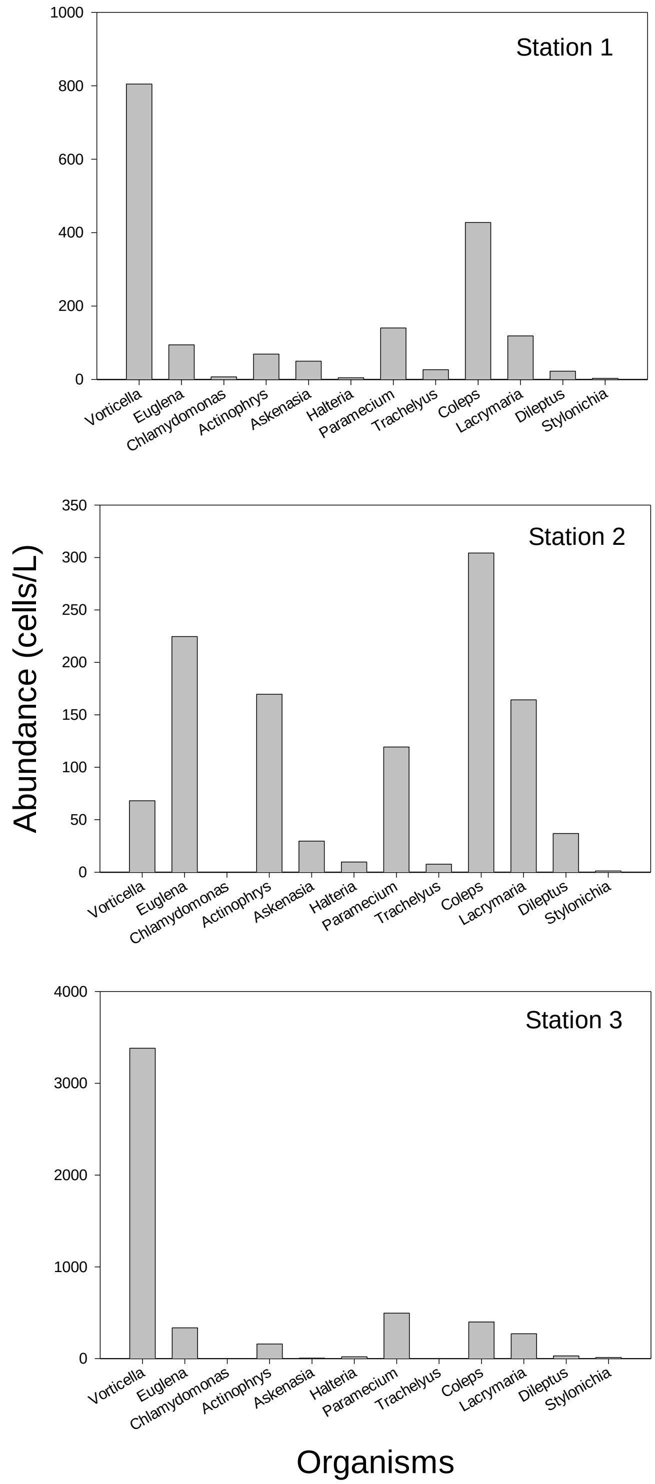

Figure 2: Abundance (%) of protists in the entrance (Station 1), middle (Station 2), and outlet (Station 3) of the secondary treatment system of the Alberto Pasqualini Oil Refinery between January and July 1996.

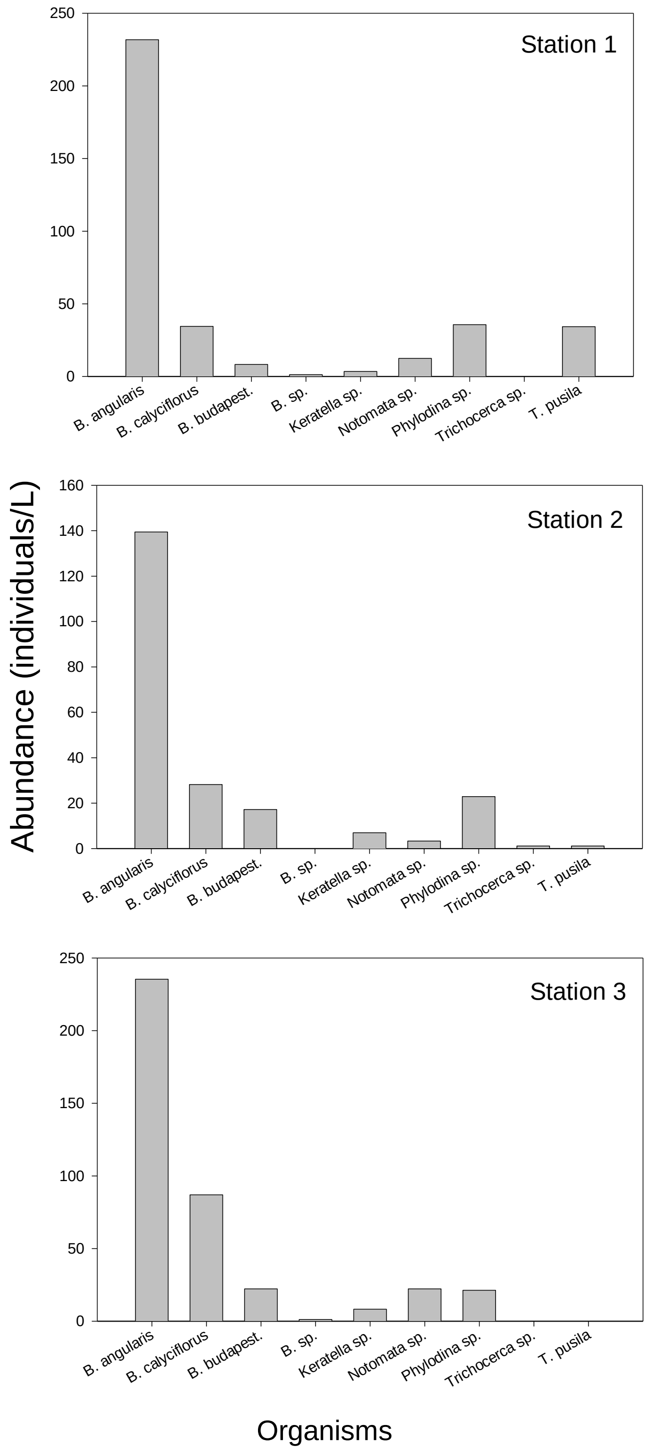

Figure 3: Abundance (%) of rotifers in the entrance (Station 1), middle (Station 2), and outlet (Station 3) of the secondary treatment system of the Alberto Pasqualini Oil Refinery between January and July 1996.

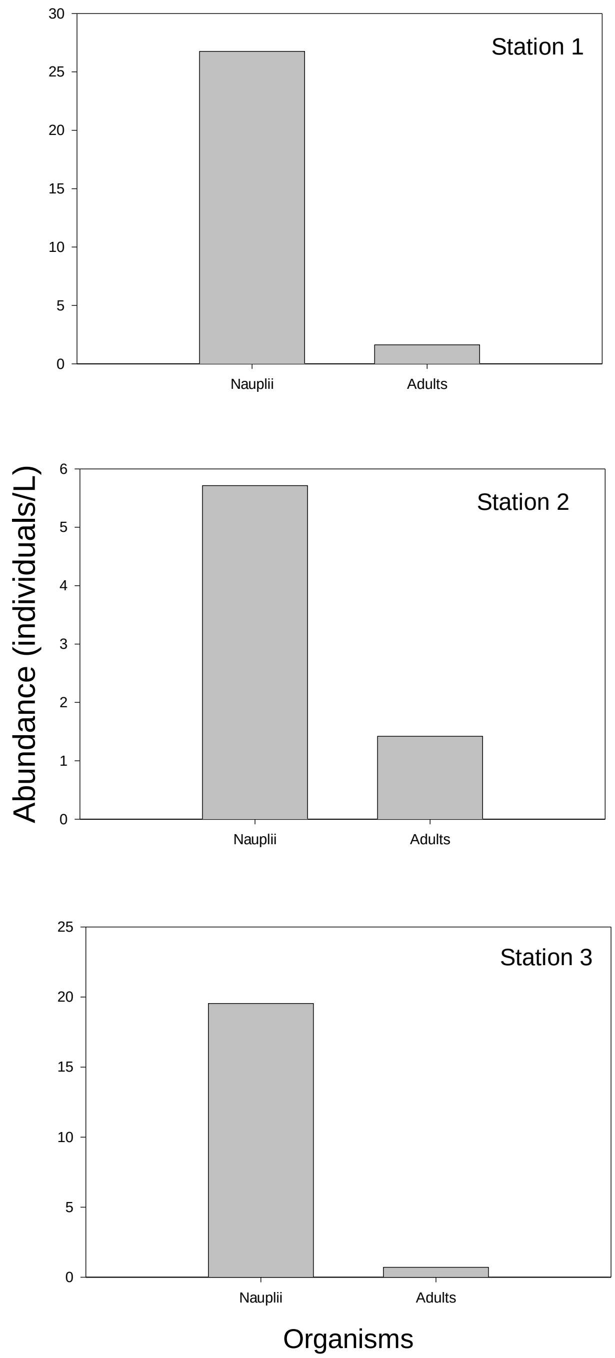

Figure 4: Abundance (%) of nauplii and adults of copepods in the entrance (Station 1), middle (Station 2), and outlet (Station 3) of the secondary treatment system of the Alberto Pasqualini Oil Refinery between January and July 1996.