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Effects of sewage water on diatoms (Bacillariophyceae) and water quality in two tropical streams in Costa Rica

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Abstract: The influence of pig farm sewage on water quality and diatoms was studied in two tropical streams over a five-month period. Sewage water inflows led to increased concentrations of soluble reactive phosphorus, ammonium, nitrite and to oxygen deficits. Due to self-purification, a decrease of ammonium coupled with an increase in nitrate occurred downstream. In total, 127 species of diatoms were recorded. Thirteen species restricted to tropical regions were found particularly at the spring-site. Correlation between dominant species and hydro-chemical parameters were calculated: *Nitzschia fonticola* occurred especially at unpolluted sites, *Gomphonema parvulum*, *Navicula subminuscula* and *Nitzschia palea* were more abundant at sewage-polluted sites and *Cocconeis placentula* seemed to be tolerant to pollution. A cluster-analysis showed that diatom assemblages at the spring-site were different from all other sites. Light and water current were discussed as factors influencing the spring-site assemblages.

Key words: Diatoms, tropical streams, water chemistry, organic pollution, nutrients.

Increases in population, deforestation, and intensified agriculture during the last decades have led in Costa Rica to the destruction and contamination of the environment, affecting among other things its freshwater systems. Sewage effluents, for instance, are usually turned directly into streams and rivers without being treated at all, and are then left to self-purification. Hence, streams and rivers are heavily polluted. According to Durán & Ocampo (1994) the daily volume of sewage and industrial effluents discharged into Costa Rican streams and rivers consists of 18% fecal pollution, 23% industrial effluents and 59% wastes from the coffee industry. Water pollution is concentrated on the Central Valley, which supports the highest population density of the country (Silva-Benavides 1994). Consequences of water quality degradation are reduction in the quantity and quality of drinking water and of recreational activities and harvest of living resources such as fishing. Additionally, the transport of contaminants and nutrients into the ocean is fast and affects marine ecosystems.

Few studies on water quality have been carried out in Costa Rican rivers. These include bacteriological and chemical surveys in rivers (Instituto Costarricence de Acueductos y Alcantarillados), chemical analysis (Cordero *et al.* 1979, Rodríguez *et al.* 1984, Ramírez 1986, Rodríguez *et al.* 1986, Pringle *et al.* 1990, Pringle 1991, Pringle & Triska 1991), heavy metal concentrations (Fuller *et al.* 1990), macrozoobenthos (Astorga-Espeleta 1994), and diatoms (Paaby 1988, Silva-Bena-

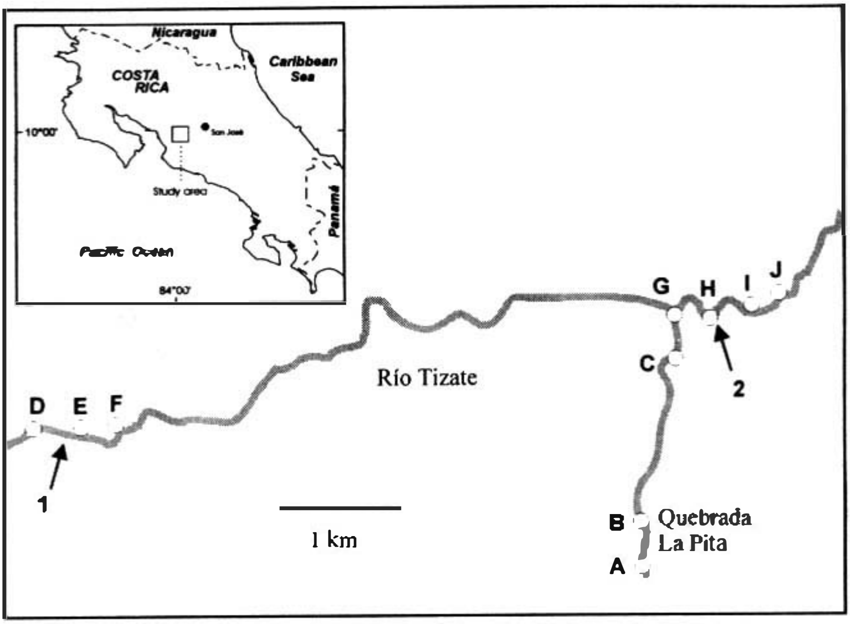

Fig. 1. Location of the sampling sites along Quebrada La Pita (A-C) and Rio Tizate (D-J). Arrow mark sewage inflows from the two pig farms. Inset: Map showing the study area in Costa Rica.

vides 1996a).

Diatoms are considered powerful indicators of water quality because they are common in all aquatic environments and all kinds of pollution levels and because they respond quickly to environmental changes (Patrick & Reimer 1966, Lange-Bertalot & Lorbach 1979, van Dam 1979, Steinberg & Schiefele 1988). Although several studies on diatoms have been carried out in the tropics (Cleve 1893, 1894, Hustedt 1937, 1938, 1939, Frenguelli 1938, Krasske 1939a, 1939b, Frenguelli 1941, Coste & Ricard 1984, Foged 1984, Coste & Ricard 1990), most of the researchers did not collect the samples personally. Thus, investigations have been restricted to taxonomy, and make no profound reference to the hydrochemical properties of the aquatic environments. Among the most important works conducted in Central America is Hagelsteins (1938) description of the diatom flora of Puerto Rico and the Virgin Islands, as well as the one of Bourelly & Manguin (1952), which comprises the diatom flora of Guadeloupe. Only the more recent investigations of Podzorski (1984, 1985) in Jamaica and Silva-Benavides (1996a) in Costa Rica, considered the hydrochemical properties of the aquatic environments. Therefore, little information exists on the ecological requirements of diatoms in the neotropics.

This paper documents the effects of sewage water on water quality and diatom flora of two streams in the Central Valley of Costa Rica. The two streams were selected with respect to different degrees of anthropogenic influence: the pollution-free Quebrada La Pita was compared with Río Tizate, polluted by pig farm sewage water.

The major objectives of this study were:

a) description of diatom species composition and the major environmental parameters of the streams;

b) correlation between the dominant species and the physico-chemical parameters;

c) detection of changes within the diatom communities due to sewage-water pollution.

MATERIALS AND METHODS

Site description: The study area was situated in the Central Valley of Costa Rica 15 km SW from Alajuela (9°50' N, 84°20' E) (Fig. 1.) near the larger town of Turrúcares at an altitude of 450-650 m a.s.l. The two investigated streams belong to the catchment basin of the Rio Grande de Tárcoles, which drains an area of 2 169 km^2, and flows into the Pacific Ocean (Fig. 1). Volcanic rocks of the upper Miocene-Pliocene, made up principally of lava flows, agglomerates and breccia, are the prevalent rock types (Tournon 1997). Climate is determined by a rainy, and a dry season. During the study period there was an annual rainfall of 2 000 mm and an average annual temperature of 24°C-27°C (Anonymous 1993).

Quebrada La Pita is an undisturbed spring-fed stream. It was sampled at three sites (A-C). Río Tizate received the sewage-waters of two pig farms, one in San Miguel (pig farm 1) and one in Cebadilla (pig farm 2). It was sampled at seven sites along four km: one site was chosen upstream the pig farms and two sites downstream and an additional site was selected, where the two streams met (sited D-J). At pig farm 1 at San Miguel 600 pigs were kept and the distance from the pig farm to the stream was 100 m. At pig farm 2 at Cebadilla 200 pigs were kept and the distance to the stream was 500 m.

Sampling: The ten sites were sampled every three weeks from February to June 1995 (16 February, 6 March, 26 March, 20 April, 10 May, 29 May and 16 June). The following samples were collected at each site: a one liter water sample in an acid-cleaned polyethylene bottle for chemical analysis, one water sample for determination of oxygen in glass-stoppered Winkler-bottles, and one diatom sample. Conductivity, temperature and pH were measured immediately in the field.

Physical parameters: Water and air temperature: were measured with a WTW conductivity meter, type LF91.

Conductivity: was measured with a WTW conductivity meter type LF91.

pH: was measured with a pH meter, pHep 2, accuracy 0.2 pH units.

Dissolved oxygen: was determined using the method of Winkler (Schwoerbel 1994).

Biochemical oxygen demand: Two 100-ml water samples were taken and the amount of dissolved oxygen was determined in one of them. The second sample was stored for 5 days in a dark incubator at 20°C, after which its dissolved oxygen was determined. The difference in the concentration of oxygen in the original sample and the stored sample represents the biochemical oxygen demand in five days (BOD_5).

Chemical parameters: Water samples were transported on ice within a eight-hours period to the laboratory, where they were filtered through Scheicher & Schüll glass fiber filters and kept frozen until chemical analysis. They were analyzed for the following parameters:

Nitrate-nitrogen: colorimetric determination with sodium salicilate and sodium hydroxide according to the method of Scheringa (DEV in Schwoerbel 1994).

Nitrite-nitrogen: colorimetric determination as a pink azo dye with sulfanilamide and N-(1-naphthyl)-ethylendiammine dihydrochloride solution (Strickland & Parsons 1972).

Ammonium-nitrogen: colorimetric determination as indophenolblue according to Berthelot (DEV in Schwoerbel 1994).

Soluble reactive phosphorus: colorimetric determination as a blue-colored complex according to the molybdenum blue technique (Strickland & Parsons 1972).

Dissolved silicate: colorimetric determination as a molybdosilicate (Strickland & Parsons 1972).

Diatom samples: As organisms are not distributed equally in their habitat, several rocks had to be sampled to obtain an adequate representation of the diatom community. At each site four to five rocks (ca. 15 cm in diameter) were collected in a water depth of 10-20 cm along a transect. The water exposed upper surfaces from the rocks were scraped carefully with a toothbrush, to make sure that all the attached algae from the various microenvironments of the rocks were considered. The scraping was transferred into a plastic vial with 50 ml of stream water and fixed with a few drops of formaline (4%). Algal samples were cleaned in the lab to oxidize the organic matter, so that just the silica cell walls of the diatoms remained. Samples were kept in H_2O_2 (30%) for at least 24 hours, then boiled for three to four hours (van der Werff 1955). To complete oxidation, a few grains of potassium dichromate were added to the mixture. To clean the samples from H_2O_2, distilled water was added and then the samples were centrifuged (2 000 U/min), for 20 minutes. This process was repeated until samples were colorless. From each diatom sample, one drop of the concentrate was placed on an alcohol-cleaned cover slip, and after air-drying, fixed with Naphrax on a slide.

At least 400 diatom valves were counted and identified from each sample on a Leitz Aristoplan microscope under 1 250 magnification under oil immersion. The diatom counts yielded relative species abundance, which is defined as the number of individuals of a given species as a proportion of the total number of individuals of all species counted in one sample.

Determination of species was done mainly according to Krammer & Lange-Bertalot (1986, 1988, 1991a, 1991b). Diatoms were presented as micrographs (Fig. 5-12), made on an Orthomat-Leitz photo-attachment, using Agfa Ortho Professional films.

Data analysis: A Spearman Rank correlation analysis was used to evaluate the relationship between changes in the abundance of the dominant species (> 10%) and physico-chemical variables.

Additionally, to observe similarity relations of diatom samples from different sampling dates and sampling sites, a minimum variance-cluster analysis was performed using the statistical program Mulva-5 (Wildi & Orlóci 1990). The Euclidean distance was used as similarity measures between two samples, species with a relative abundance of less than 3% were excluded.

RESULTS

Physico-chemical parameters: Average values and standard deviation for all physico-chemical parameters except temperature and BOD_5 are summarized in Fig. 2.

Air and water temperature: Both parameters didn't vary much during the sampling period. Water temperatures were generally in the range 21.1°C-26.4°C, mean water temperature of all sites was 24.2 °C. Air temperatures were in the range 21.6°C –29.5°C with an average of 24.7°C.

pH (Fig. 2): With the exception of the spring-site (A), the pH generally showed a slightly alcaline character of the water, finding values between 7.2 – 8.0 pH. Just at sites which were highly sewage influenced (sites E, F) a less alcaline pH, closed to the neutral point of 7 pH units was found.

Conductivity (Fig. 2): Conductivity showed a similar pattern for every sampling data: almost constant values (152-158 µS/cm) at sites along Quebrada La Pita (sites A, B, C), a sudden rise downstream pig farm 1 at sites E, F (252-542 µS/cm) along Río Tizate and a smaller rise downstream pig farm 2 at sites I, J (208-249 µS/cm).

Dissolved oxygen (Fig. 2): Oxygen deficits, which are typical for springs, occurred at the spring-site A; downstream oxygen concentration were near or above saturation (sites B, C). The sites on Río Tizate downstream the pig farms (sites E, F, I, J) showed low oxygen contents. At sites G and H oxygen levels reached saturation.

Biochemical oxygen demand (BOD_5): Because of logistic problems BOD_5 could only be measured at two occasions (6 March 1995, 29 May 1995). BOD_5 was low (0-1.1 mg/l) at unpolluted and intermediate sites A, B, C, D, G, and H. Downstream the pig farms, at sites E, F, I, and J BOD_5 increased, wirh highest values of 4.5-7 mg/l downstream pig farm 1.

Dissolved silica (Fig. 2): Silica is the essential element for diatoms and was relatively abundant at all sites always reaching concentrations higher than the limiting concentration of 2 mg/l (Klee 1991). Variations were high within a site and among sites during the whole sampling period.

Soluble reactive phosphorus (SRP) (Fig. 2): Unusually high concentrations of 148-227 µg/l SRP were found at the undisturbed sites on Quebrada La Pita (sites A-C). Highest levels of SRP (790-2 697 µg/l) were measured at sampling sites downstream the pig farms.

Nitrogen (Fig. 2): Nitrite and ammonia were found in higher concentrations (nitrite: 14-89 µg/l, ammonia 0.1-2.2 mg/l) right after the sewage inflows at sites E, F, I and J. Nitrate levels were generally low with concentrations of 0.3–0.5 mg/l at the unpolluted sites. At site G nitrate concentrations increased to 1.0-1.8 mg/l.

Diatoms: 127 species belonging to 27 genera were identified and documented (Fig. 5-12). Thirteen species could only be determined to genus level because they were to

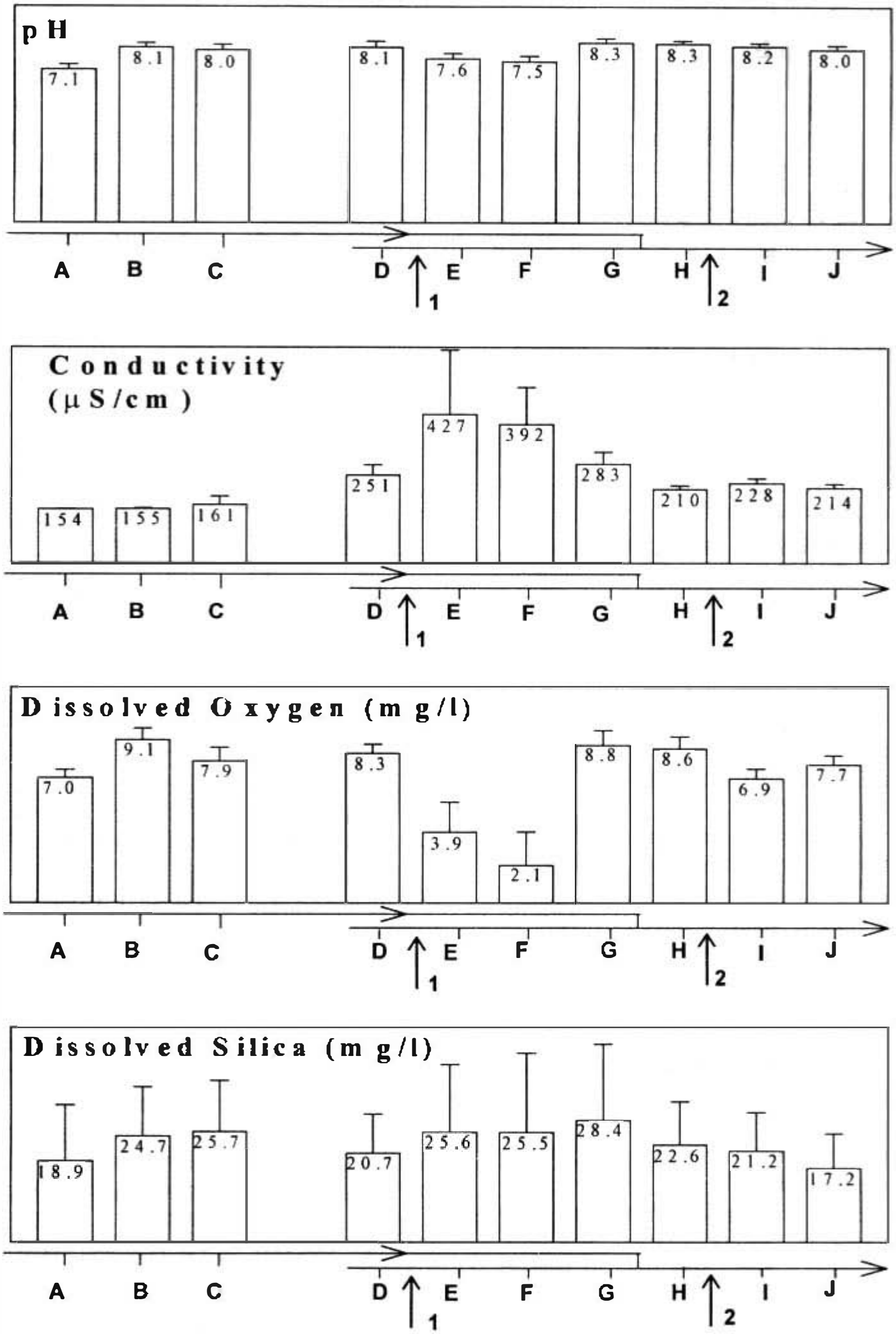

Fig. 2. Part 1. Physico-chemical parameters (average values, standard deviation, n=7) at the sampling sites along Quebrada La Pita (A-C) and Rio Tizate (D-I) in the period February to June 1995. Arrows mark sewage inflows from the two pig farms.

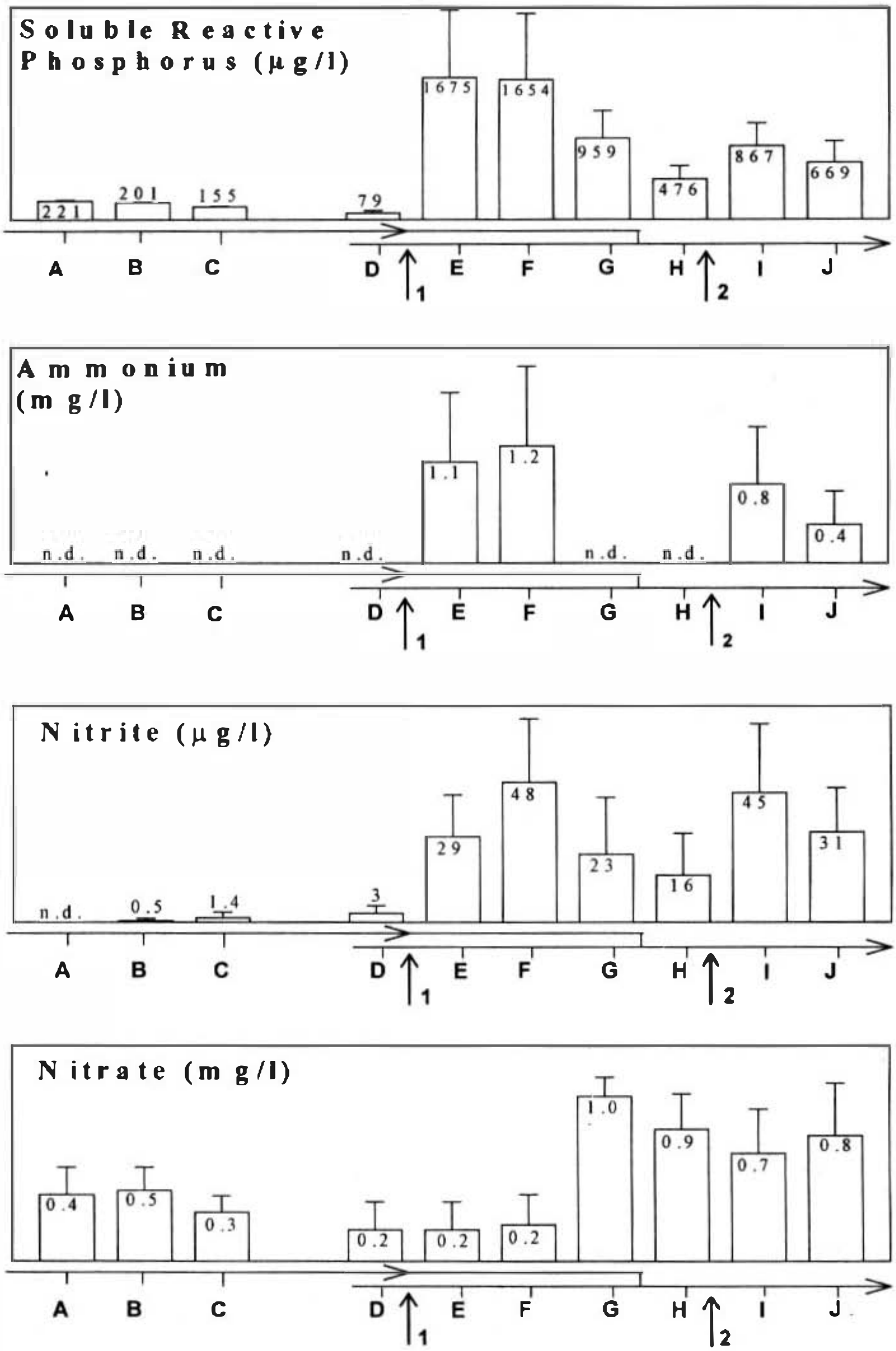

Fig. 2. Part 2. Physico-chemical parameters (average values, standard deviation, n=7) at the sampling sites along Quebrada La Pita (A-C) and Rio Tizate (D-J) in the period February to June 1995. Arrows mark sewage inflows from the two pig farms.

small (< 10 µm) for good microscopic resolution, and so rare that there were not enough specimens for reliable identification.

On the basis of geographical distribution, thirteen species out of the 127 recorded in this study appear to prefer tropical areas according to literature (Hustedt 1937, 1938, 1939, Krammer & Lange-Bertalot 1986, 19881, 1991a, 1991b, Reichardt 1988a, Coste & Ricard 1990). These species listed in Table 1 made up 15.8% of the entire diatom community.

TABLE I

Species with preference to tropical areas found at the sampling sites along Quebrada La Pita (A-C) and Rio Tizate (D-I) in the period February to June 1995 (n=7)

Achnanthes praecipua Reichardt
Achnanthes salvadoriana Hustedt
Cymbella turgidula Grunow
Eunotia camelus Ehrenberg
Diploneis subovalis Cleve
Fragilaria goulardii (Brébisson) LangeBertalot
Gomphonema mexiconum Grunow
Gomphonema parvulum var. lagenula Kützing
Hantzschia sigma Hustedt
Navicula chilena Lange-Bertalot
Navicula confervacea Kützing
Navicula ruttneri Hustedt
Navicula ruttneri var. chilensis Krasske

The majority of the species (83 out of 127) were never present with abundance of more than 3%. The remaining 45 species, that reached abundance of more than 3%, comprised at least 80% of the diatom community of each sample and in total 91.85% of the entire diatom community found in this study. The most common species were *Achnanthes praecipua, Amphipleura lindheimerii, Cocconeis placentula, Gomphonema parvulum, Gomphonema parvulum var. lagenula, Navicula erifuga, Navicula ruttneri, Navicula seminulum, Navicula subminuscula, Nitzschia amphibia, Nitzschia fonticola, Nitzschia frustulum, Nitzschia linearis* and *Nitzschia palea.*

In Fig. 3 the abundance of a few selected species, described below in more detail, are shown:

Achnanthes praecipua Reichardt was the most common species at the spring-site A (Fig. 3). No significant correlation was found between the physico-chemical parameters and the relative abundance of this species.

Cocconeis placentula Ehrenberg was the quantitatively most important species of this study (17.3% of the entire diatom community). Except for the spring-site this species was represented at all sampling sites with relative abundance of >10% (Fig. 3). No significant correlation was found between the physico-chemical parameters and the relative abundance of this species.

Gomphonema parvulum Kützing was found in very abundant populations with mass development (max. 49% at site E) only at sites affected by the sewage water (Fig. 3). In this study negative correlation were found to oxygen ($p<0.0001$, $r_S=-0.42$) and positive to SRP ($p<0.0001$, $r_S=+0.46$), ammonium ($p<0.001$, $r_S=+0.48$), nitrite ($p<0.0001$, $r_S=+0.51$) and conductivity ($p<0.002$, $r_S=+0.3613$).

Navicula ruttneri Hustedt occurred in relative abundance of more than 10% only at the spring-site (Fig. 3). In this study negative correlation were found to conductivity ($p<0.0001$, $r_S=-0.58$), nitrite ($p<0.001$, $r_S=-0.46$) and ammonia ($p<0.0001$, $r_S=-0.51$).

Navicula seminulum Grunow was represented all along the sampling sites and particularly highly abundant (13.6-39.7%) at the spring-site A (Fig. 3). In this study no significant correlation between the physico-chemical parameters and the relative abundance of this species were found.

Navicula subminuscula Manguin was found in high relative abundance with mass development (max. 47.1% at site I) only at sewage affected sites (Fig. 3). In this study positive correlation were found to conductivity ($p<0.0001$, $r_S=+0.46$), nitrite ($p<0.001$, $r_S=+0.52$) and negative correlation to oxygen ($p<0.0001$, $r_S=-0.4406$).

Nitzschia fonticola Grunow reached high percentages (10%-20%) only at sites B and C downstream the spring (Fig. 3). In this study a positive correlation to oxygen ($p<0.0001$, $r_S=+0.44$) was calculated.

Nitzschia palea Smith was presented in low densities throughout the study period. At sewage affected sites it occurred in higher relative abundance of 5%-9% (Fig. 3). In this study positive correlation were found to ammonia ($p<0.0001$, $r_S=+0.51$) and SRP ($p<0.001$, $r_S=+0.40$).

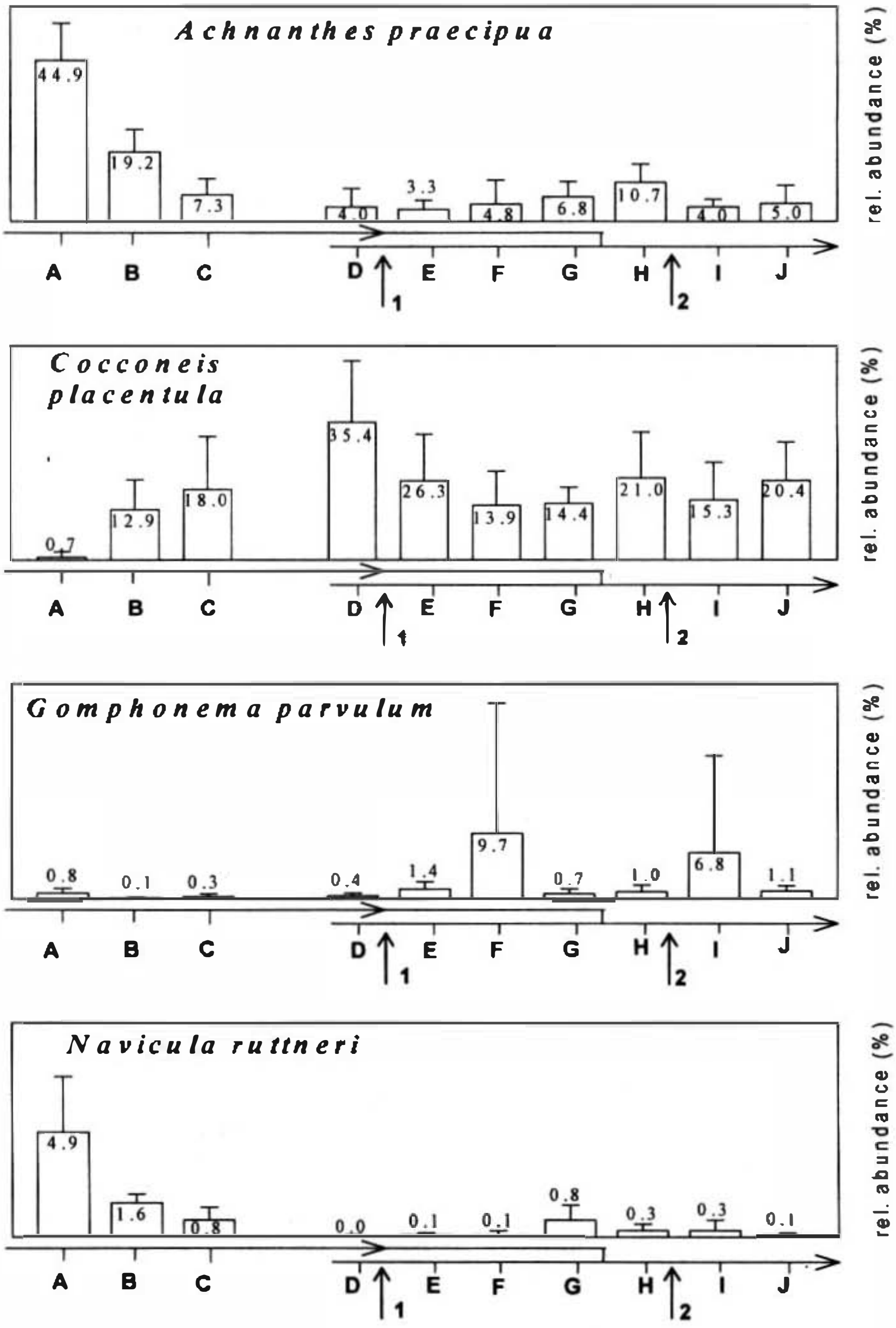

Fig. 3. Part I. Relative abundances (average values, standard deviation, n=7av ± sd) of some of the dominant species at the sampling sites along Quebrada La Pita (A-C) and Río Tizate (D-I) in the period February to June 1995. Arrows (↑) mark sewage inflows from the two pig farms.

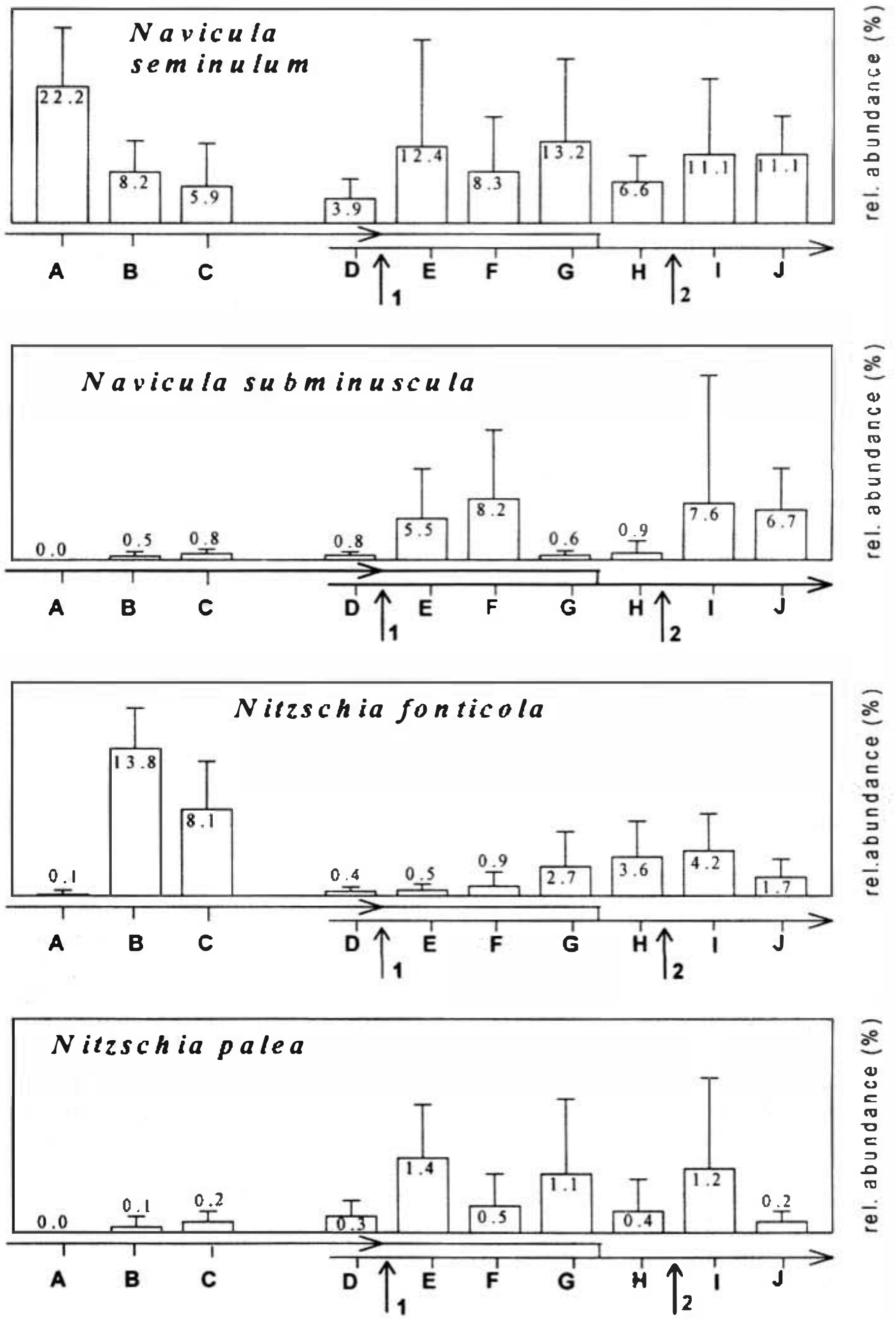

Fig. 3. Part 2. Relative abundances (average values, standard deviation, n=7av ± sd) of some of the dominant species at the sampling sites along Quebrada La Pita (A-C) and Río Tizate (D-I) in the period February to June 1995. Arrows (↑) mark sewage inflows from the two pig farms.

In general the correlation between physico-chemical parameters and species abundance were not strong, most species tested had an r_s of 0.5 or lower.

Cluster analysis: The dendrogram (Fig. 4) can be divided into three groups:

1) Group I was separated at the highest division level, consisting exclusively of samples from the spring-site (A);

2) Group II contained sites of Quebrada La Pita (B, C);

3) Group III was formed by sites from Rio Tizate (sites D to J). Group III can be subdivided into four subgroups:

a. sewage affected sites E, F, and I;
b. site D;
c. sites between the two pig farms (G, H) and some sewage affected sites (E, F, I, J) and
d. mainly sewage affected sites (E, F, I, J).

Grouping of sampling sites did occur mainly according to streams and most closely related stream sections.

DISCUSSION

Biogeographical remarks: Most species found in this study can be considered as cosmopolitan. Several other investigations conducted in tropical regions have observed similar proportions of cosmopolitan species as in this study: 90% by Silva-Benavides (1994) in Costa Rica, 90% by Podzorski (1984) in Jamaica and 80% by Reichardt (1988b) in Papua New-Guinea. Thus, use can be made of taxonomic and ecological studies carried out in temperate zones.

In addition to cosmopolitan species, there are diatom species, which prefer tropical regions. An important reason for this distribution are climatic and hydrological conditions (Krammer & Lange-Bertalot 1986), but the role of each of this components is not truly understood. The distribution of most tropical diatom species is almost unknown, most descriptions found in the literature are based on unique samplings (Cholnoky 1968, Krammer & Lange-Bertalot 1986).

In this study almost all of the 13 species (Table 1) with preference to tropical regions were restricted to the spring-site, whereas polluted sites were dominated by cosmopolitan species. These results support the hypothesis made by Silva-Benavides (1994) that species preferring tropical regions are found particularly at pristine sites. This may be because some exclusive ecological conditions give tropical species competition advantages over cosmopolitan species at undisturbed tropical sites. Anthropogenic impact may change these exclusive ecological conditions with organic wastes, deficiency in oxygen, high nutrient inputs, for example. If this were true, then species restricted to the tropics would decline compared with cosmopolitan species at disturbed sites. Future studies should focus on the diatom communities in undisturbed tropical regions, to get a better understanding of the factors favoring the occurrence of tropical diatoms species.

The spring-site (A): As can be seen from the cluster-analysis (Fig. 4) the spring-site was a notable exception during the course of this study. This site was dominated by few species represented in high abundance. Which are the factors making the spring-site distinctive? The mass-development of few species in springs has been linked to factors like oxygen, nutrient chemistry or temperature (Budde 1928, Hustedt 1937, 1938, Cholnoky 1966). In the present study however, none of the above mentioned parameters fit, because these variables were very similar at the spring-site and at the sites downstream the spring-site. Additional variables were light intensity and current velocity. The spring-site was surrounded by high trees and rocks, had deep shade, and there was almost no water movement. Several studies demonstrated that light intensity is an important factor affecting the distribution and abundance of algae (McIntire & Phinney 1965, Keithan & Lowe 1985, Robinson & Rushford 1987). Steinman & McIntire (1986) noted in laboratory experiments, that some species, i.e. *Achnanthes lanceolata* and *Synedra ulna* developed more successfully under low light levels than other species, suggesting that some algal species are "shade-adapted". Experimental studies have also shown that current velocity is an important variable influencing the distribution and abundance of diatoms. Many species have been found to occupy specific flow regimes (Allan 1995). Keithan & Lowe (1985) found species of *Achnanthes* to be most abundant in fast current communities, Kuhn *et al.* (1981) noted that *Achnanthes minutissima* predominated on

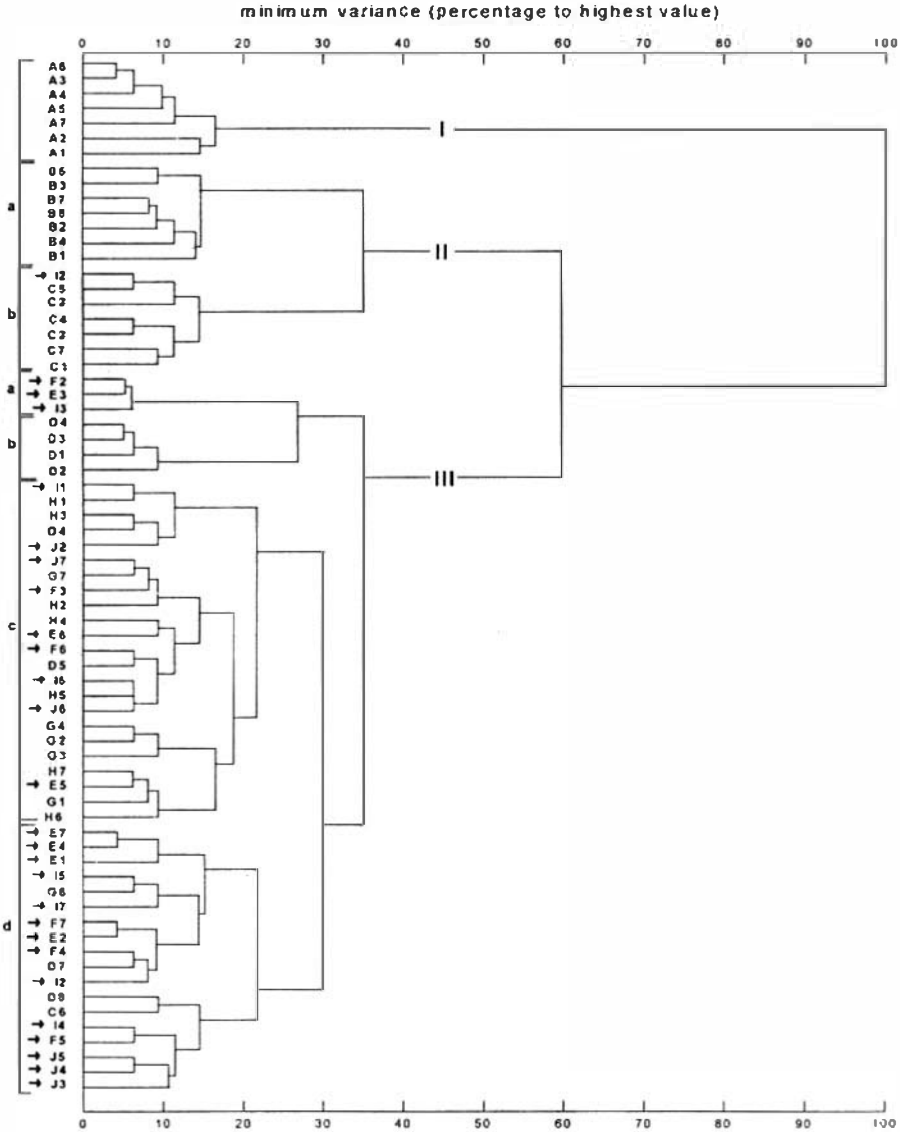

Fig. 4. Dendrogram showing similarity relations of epilithic diatoms at the 10 sampling sites along Quebrada la Pita (A-C) and Río Tizate (D-I) for seven sampling dates (1-7 = chronological order). Only species with relative abundances of >3% were included. Arrows (↑) mark sewage inflows from one of the two pig farms.

highly disturbed substrates. In the present study, however, *Achnanthes* species were most common at the spring-site, where there was almost no water movement. Experiments examining the effects of both light intensity and current velocity on species composition of periphyton assemblages showed that at low light intensities effects of current velocity were negligible (McIntire 1968). Similar results have been reported by Robinson & Rushford (1987). Therefore, in the present study it is more likely, that low light intensity was the cause for the predominance of *Achnanthes praecipua* and *Navicula seminulum* (>40%) at the spring-site, suggesting that these species were tolerant to low light intensities. The constant occurrence of some species with very similar abundance over the whole sampling period indicated stable conditions at the spring-site.

Effect of the sewage inflow on water quality:

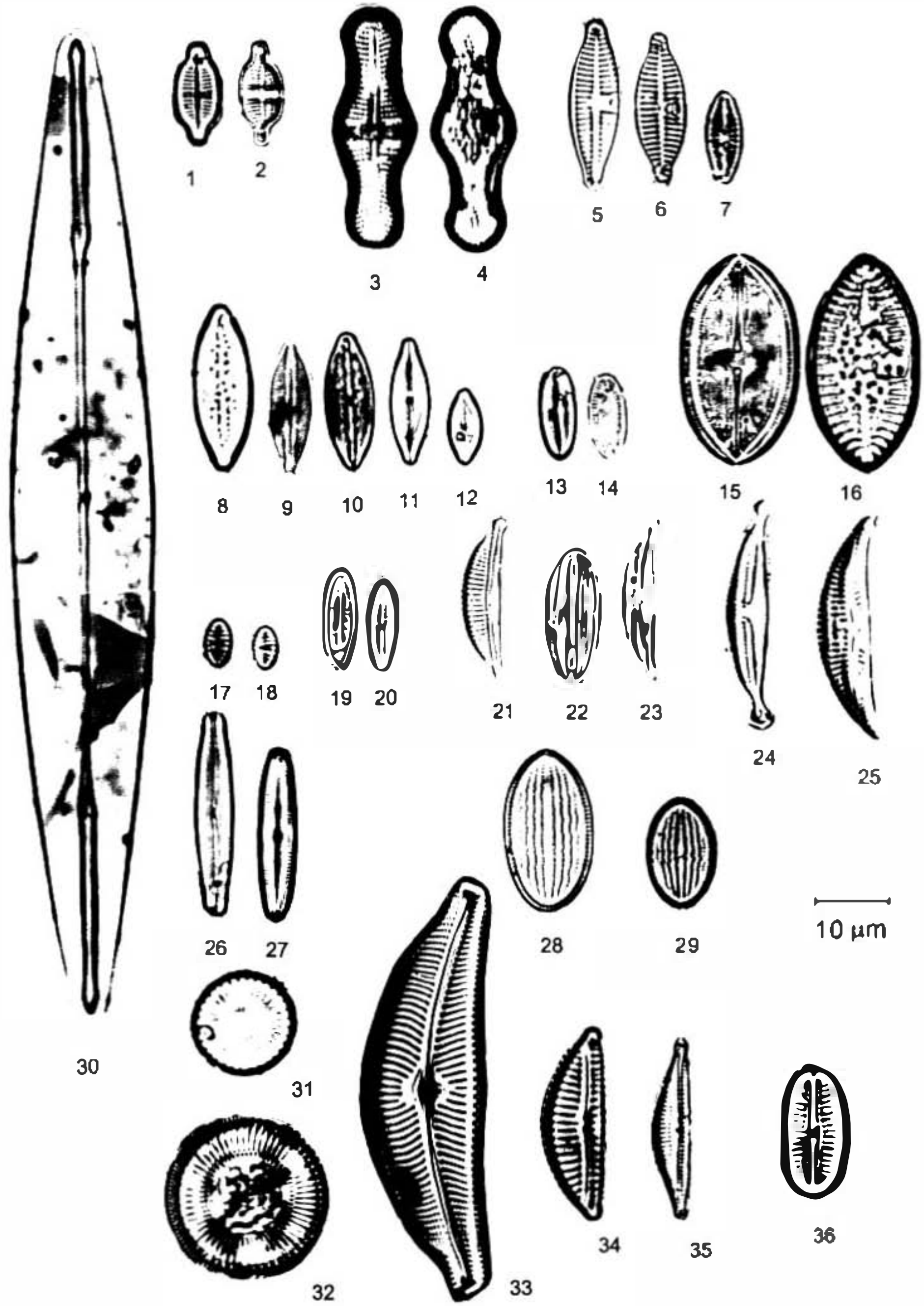

Fig. 5. 1-2. *Achnanthes exigua* Grunow; 3-4. *Achnanthes inflata* Kützing; 5-7. *Achnanthes lanceolata var frequentissima* Lange-Bertalot; 8-12. *Achnanthes proecipua* Reichardt; 13-14. *Achnanthes c.f. rupestoides* Hohn; 15-16. *Achnanthes salvadoriana* Hustedt; 17-18. *Achnanthes* sp.; 19-20. *Achnanthes subhudsonis* Hustedt; 21. *Amphora acutiuscula* Kützing; 22-23. *Amphora montana* Krasske; 24. *Amphora normanii* Rabenhorst; 25. *Amphora ovalis* Kützing; 26-27. *Caloneis bacillum* Grunow; 28-29. *Cocconeis placentula* Ehrenberg; 30. *Amphipleura lindheimerii* Grunow; 31. *Cyclotella menenghiniana* Kützing; 32. *Cyclotella striata* Grunow; 33. *Cymbella tumida* Brébisson; 34. *Cymbella silesiaca* Bleisch; 35. *Cymbella* sp.; 36 *Diploneis subovalis* Cleve.

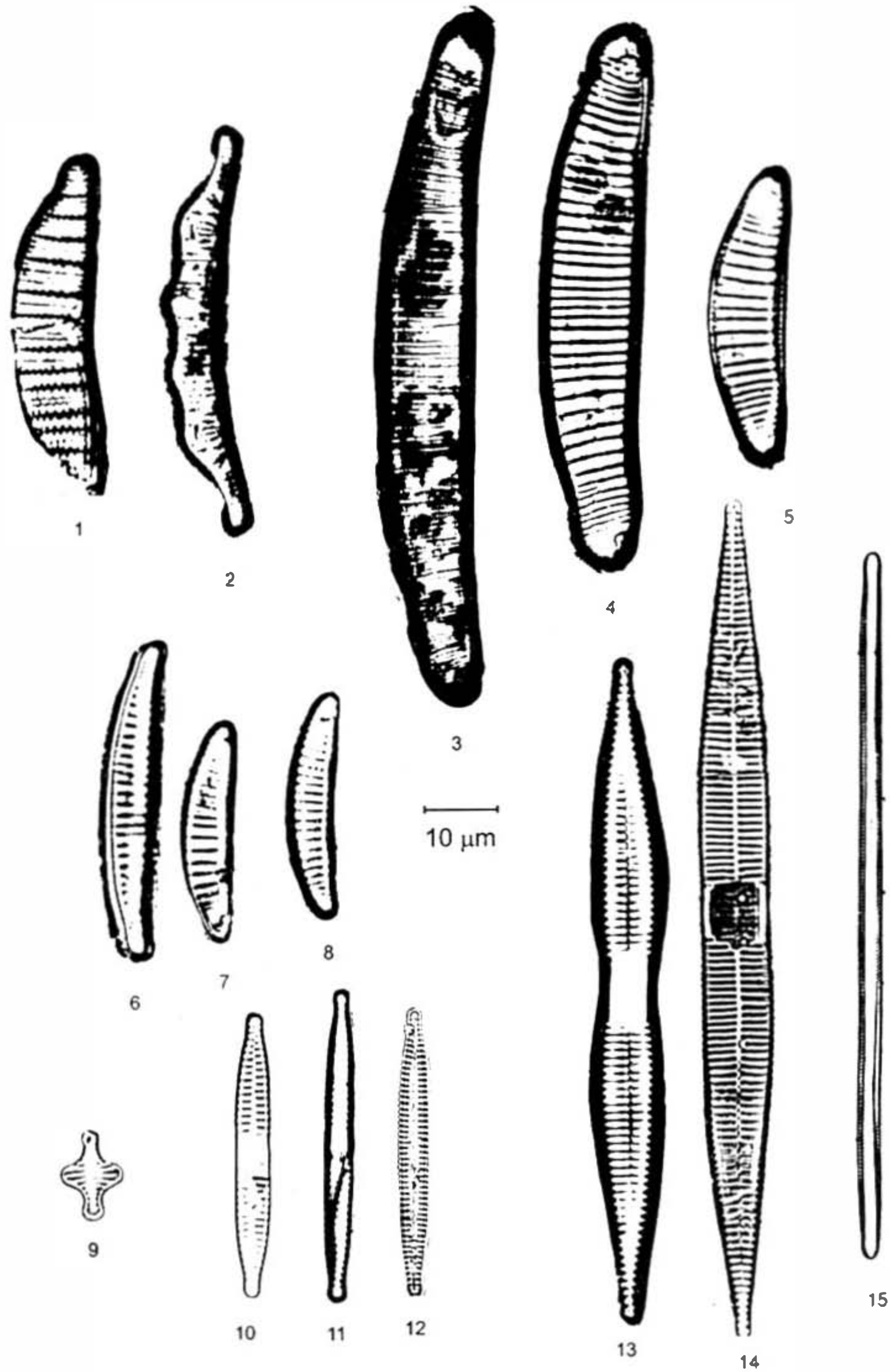

Fig. 6. 1. *Epithemia* sp.; 2. *Eunotia camelus* Ehrenberg; 3-5. *Eunotia monodon* Ehrenberg; 6-8. *Eunotia pectinalis* Rabenhorst; 9. *Fragilaria construens* Ehrenberg (Grunow); 10-11. *Fragilaria capucina var. vaucheriae* (Kützing) Lange-Bertalot; 12. *Fragilaria fasciculata* (Agardh) Lange-Bertalot; 13-14. *Fragilaria goulardii* (Brébisson) Lange-Bertalot; 15. *Fragilaria ulna* (Nitzsch) Lange-Bertalot (260 x).

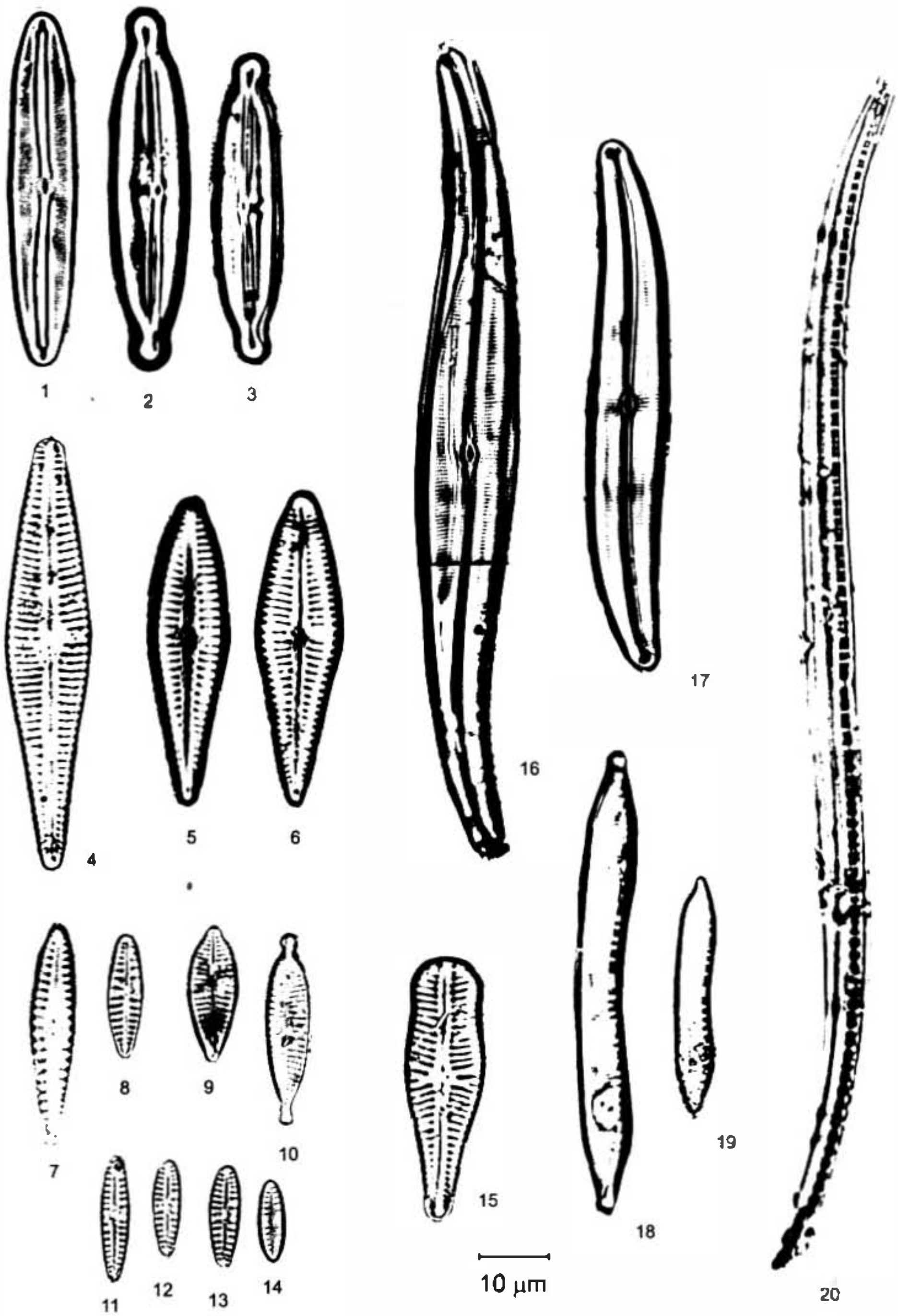

Fig.7. 1. *Frustulia weinholdii* Hustedt; 2-3. *Frustulia rhomboides* Ehrenberg; 4. *Gomphonema gracile* Ehrenberg; 5-6. *Gomphonema mexicanum* Grunow; 7-8. *Gomphonema parvulum* Kützing; 9-10. *Gomphonema parvulum var. lagenula* Kützing; 11-14. *Gomphonema pumilum var. rigidum* Reichardt; 15. *Gomphonema truncatum var. capitatum* Ehrenberg; 16. *Gyrosigma scalproides* Rabenhorst; 17. *Gyrosigma strigilis* Cleve; 18-19. *Hantzschia amphioxys* Ehrenberg; 20. *Hantzschia sigma* Hustedt.

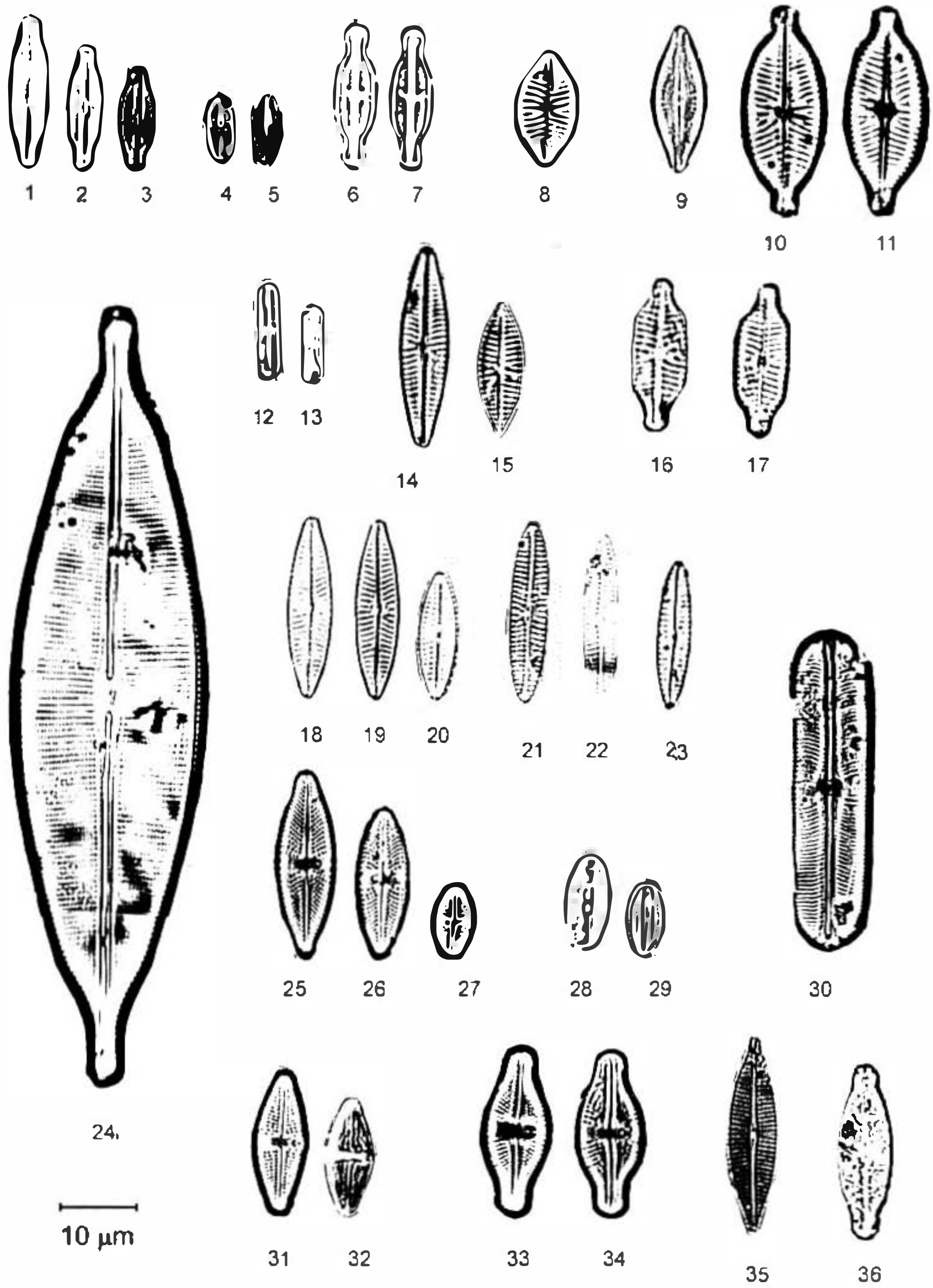

Fig.8. 1-3. *Navicula arvensis* Lange-Bertalot; 4-5. *Navicula atomus* Kützing; 6-7. *Navicula chilena* Lange-Bertalot; 8. *Navicula clementis* Grunow; 9. *Navicula confervacea* Kützing; 10-11. *Navicula constans var. symmetrica* Hustedt; 12-13. *Navicula contenta* Grunow; 14-15. *Navicula cryptocephala*; 16-17. *Navicula elginensis*-Artengruppe Ralfs; 18-20. *Navicula erifuga* Sippe1 Lange-Bertalot; 21-23. *Navicula c.f. erifuga* Sippe 2 Lange-Bertalot; 24. *Navicula cuspidota* Kützing; 25-27. *Navicula goeppertiana* (Bleisch) Smith; 28-29. *Navicula insociabilis* Krasske; 30. *Navicula laevissima var. laevissima* Kützing; 31-32. *Navicula c.f. mutica var. intermedia* Kützing; 33-34. *Navicula mitigata* Hustedt; 35. *Navicula phyllepta* Kützing; 36. *Navicula c.f. protracta* (Grunow) Cleve.

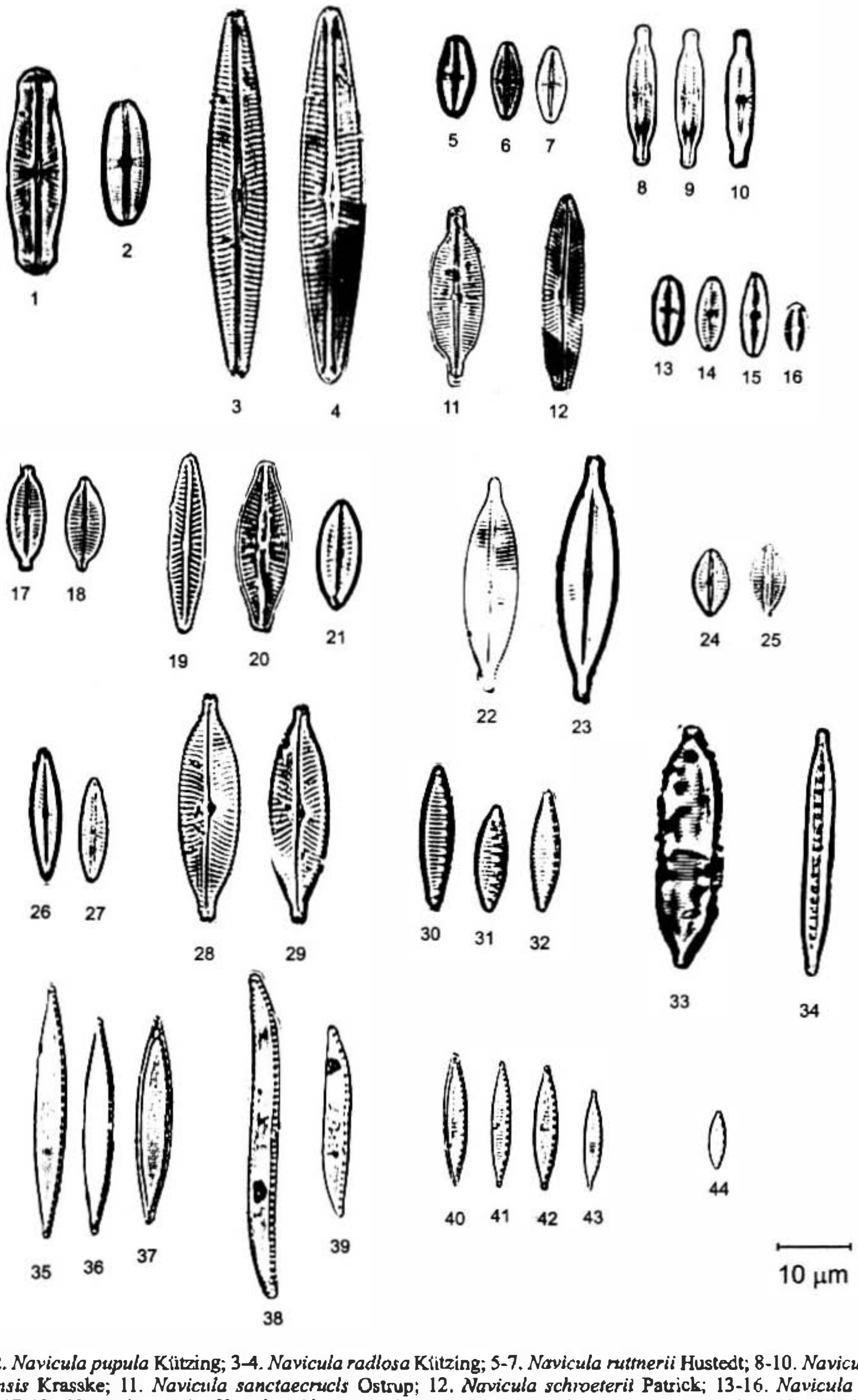

Fig. 9. 1-2. *Navicula pupula* Kützing; 3-4. *Navicula radiosa* Kützing; 5-7. *Navicula ruttnerii* Hustedt; 8-10. *Navicula ruttnerii var. chilensis* Krasske; 11. *Navicula sanctaecrucis* Ostrup; 12. *Navicula schroeterii* Patrick; 13-16. *Navicula seminulum* Grunow; 17-18. *Navicula similis* Krasske; 19. *Navicula* sp.1; 20. *Navicula* sp.2; 21. *Navicula* sp.3; 22-23. *Navicula subrynchocephala* Hustedt; 24-25. *Navicula subminuscula* Manguin; 26-27. *Navicula tenelloides* Hustedt; 28-29. *Navicula viridula var. rostellata* Cleve; 30-32. *Nitzschia amphibia* Grunow; 33. *Nitzschia calida* Grunow; 34. *Nitzschia dissipata* Grunow; 35-37. *Nitzschia fonticola* Grunow; 38-39. *Nitzschia clausii* Hantzsch; 40-43. *Nitzschia frustulum* Grunow; 44. *Nitzschia inconspicua* Grunow.

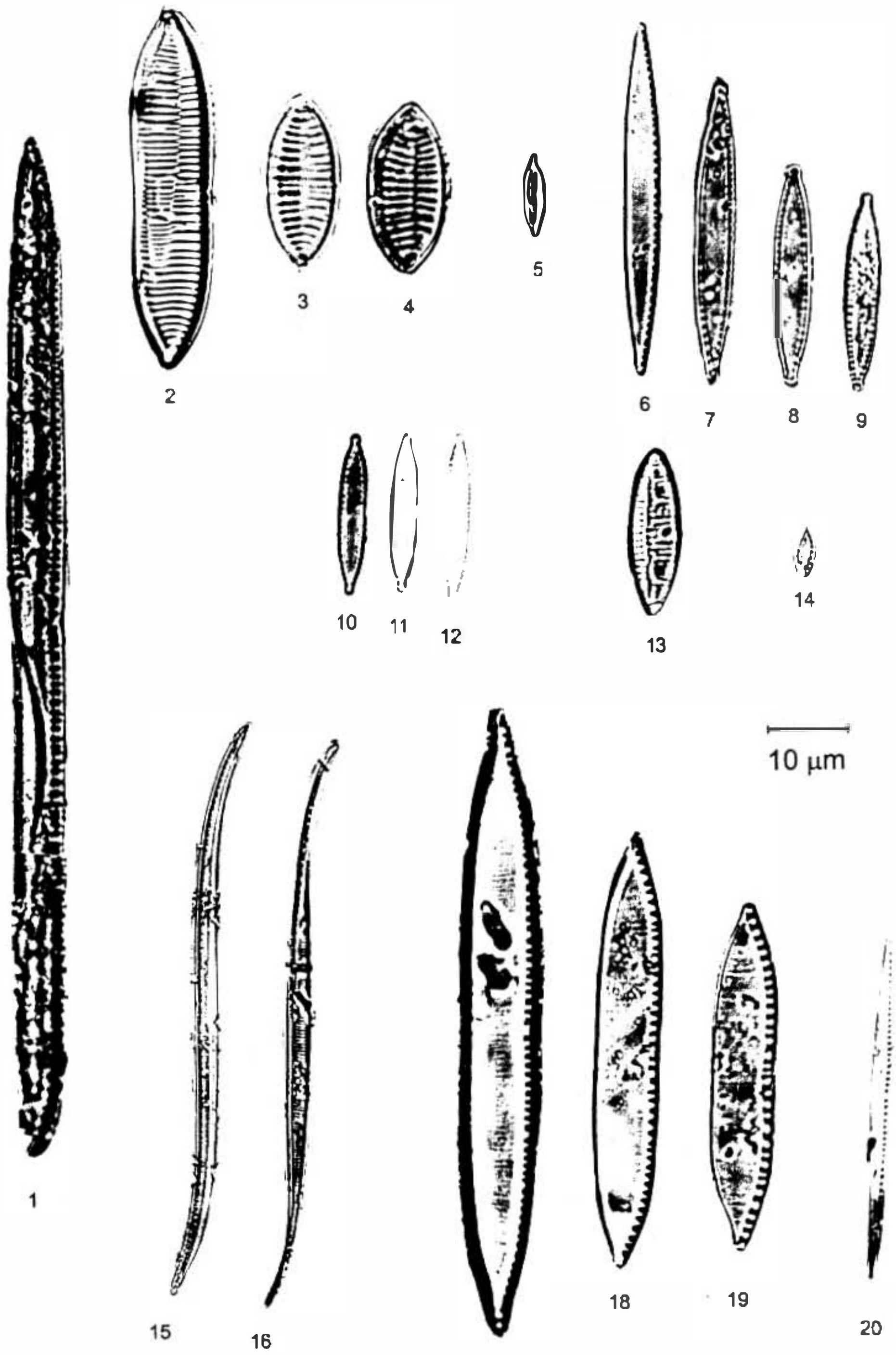

Fig. 10. 1. *Nitzschia linearis* Smith; 2-4. *Nitzschia levidensis* Grunow; 5. *Nitzschia microcephala* Grunow; 6-9. *Nitzschia palea* Smith; 10-12. *Nitzschia palea var. debilis* Grunow; 13. *Nitzschia sinuata* Grunow; 14. *Nitzschia* sp.; 15-16. *Nitzschia c.f. lorenziana* Grunow 17-19. *Nitzschia umbonata* Lange-Bertalot; 20. *Nitzschia paleacea* Grunow.

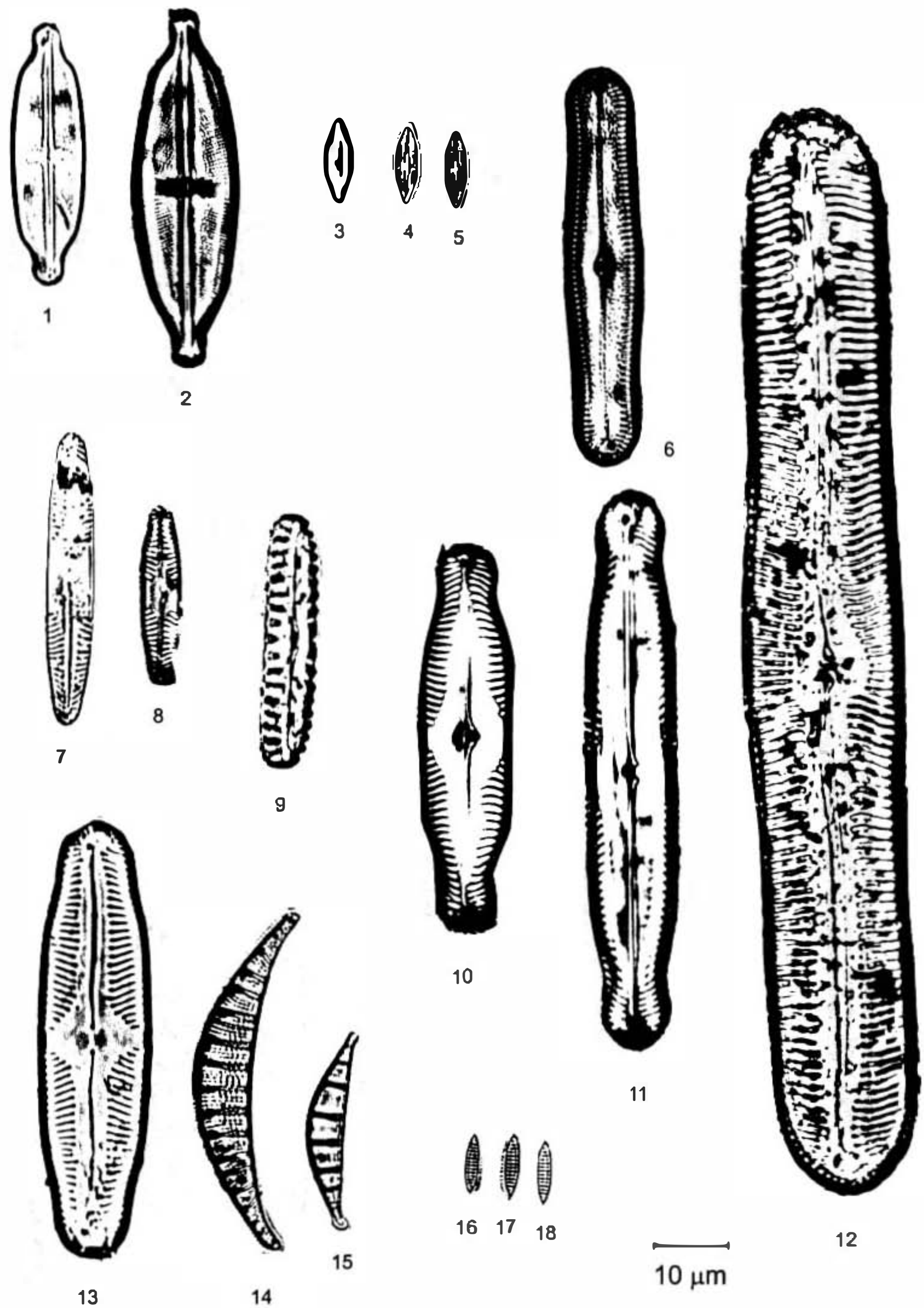

Fig. 11. 1. *Neidium ampliatum* (Ehrenberg) Krammer; 2. *Stauroneis anceps var. c.f. Sippe 2* Reichardt; 3. *Nupela ?* sp. *1*; 4-5. *Nupela ?* sp. 2; 6. *Pinnularia acrosphaeria* Rabenhorst; 7. *Pinnularia braunii* (Grunow) Cleve; 8. *Pinnularia c.f. acoricola* Hustedt; 9. *Pinnularia borealis* Ehrenberg; 10. *Pinnularia* sp. 1; 11. *Pinnularia* sp. 2; 12. *Pinnularia c.f. tropica* Hustedt; 13. *Pinnularia c.f. gibba* Ehrenberg; 14. *Rhopalodia gibberula;* 15. *Rhopalodia rupestris* (W. Smith) Krammer; 16-18. *Simensonia delognei* Lange-Bertalot.

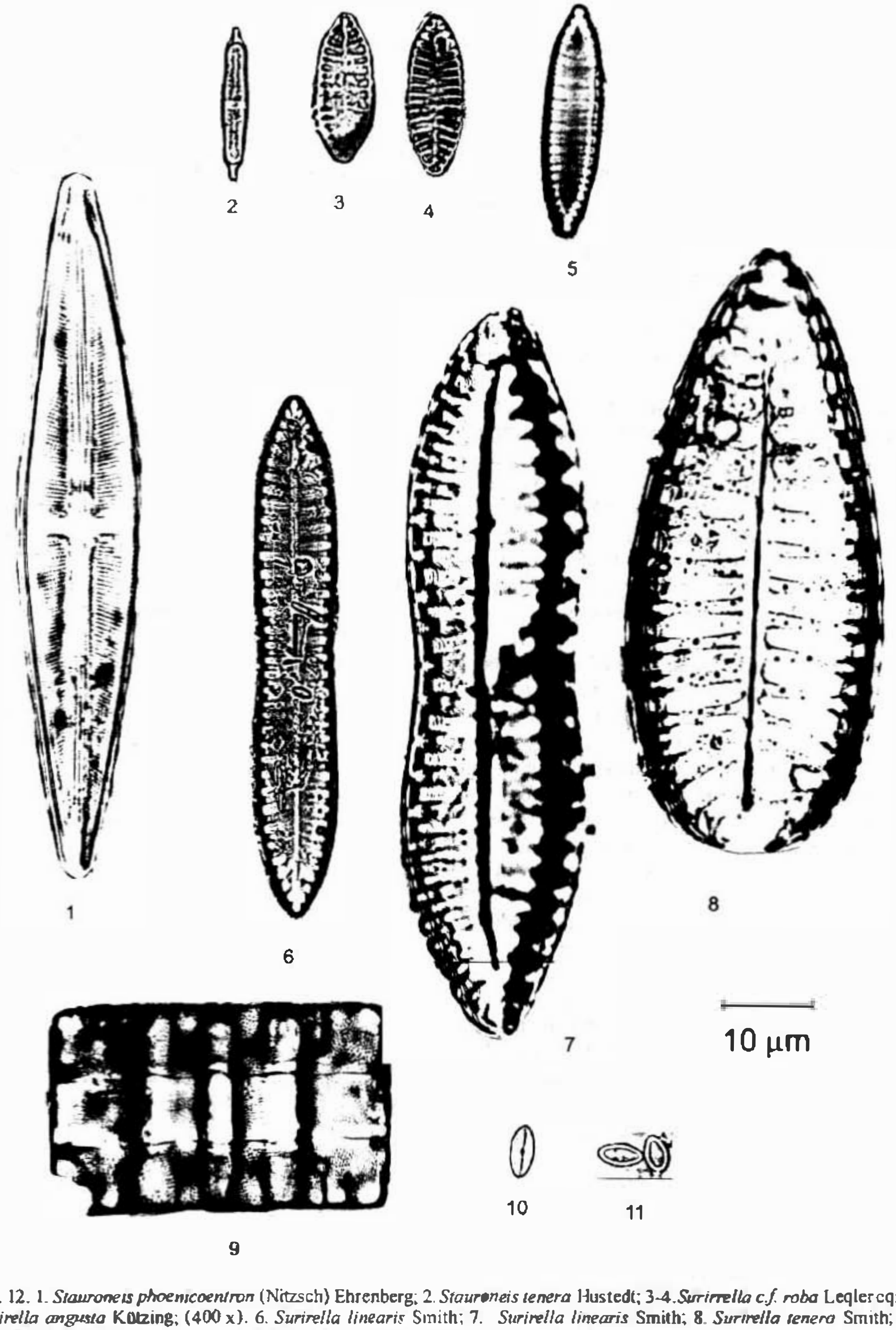

Fig. 12. 1. *Stauroneis phoenicoentron* (Nitzsch) Ehrenberg; 2. *Stauroneis tenera* Hustedt; 3-4. *Surirrella c.f. roba* Leqlercq; 5. *Surirella angusta* Kützing; (400 x). 6. *Surirella linearis* Smith; 7. *Surirella linearis* Smith; 8. *Surirella tenera* Smith; 9. *Trëpsineu musica* Ehrenberg; 10. sp. 1; 11. sp. 2.

As the best parameter for showing the effect of pollution on water quality in running water, dissolved oxygen reflected clearly the loading with organic wastes. At site G, approximately four km away from the sewage influx, oxygen levels were totally replenished as a result of intensive self-purification.

The high SRP concentrations measured at all sampling sites suggested a high background of this nutrient. This is often reported from streams that drain volcanic regions and is possibly due to weathering of young volcanic rocks (Talling & Talling 1965, Pringle *et al.* 1990, Pringle 1991, Pringle & Triska 1991, Pringle *et al.* 1993, Silva-Benavides 1994). The high SRP values were reflected also by the diatom flora, which was characterized by the presence of species that indicate eutrophic conditions according to Lange-Bertalot (1978), Steinberg & Schiefele (1988), Hofmann (1994), and van Dam (1994). The high quantities of SRP measured downstream the sewage water inflows were probably due to the decomposition of organic molecules (nucleotides, proteins) to phosphate (Schönborn 1992).

Nitrite and ammonia, could be considered as good indicators of sewage water contamination in this study. Both occurred in higher concentrations right after the sewage water inflow. Due to the conversion of ammonium to nitrate, at site G, approximately four km away from the sewage influx, an increase in nitrate concentrations was measured.

The introduction of sewage water from the pig farms induced changes in water quality of the sites downstream: increases in conductivity, BOD_5 , soluble reactive phosphorus, and ammonium nitrogen, and consequently decrease of oxygen levels. The sewage inflow at San Miguel (pig farm 1) had a much stronger effect on water quality at sites E and F, than the inflow at Cebadilla (pig farm 2) at I and J. The overall effects of pollution on water quality depended on the size of the pig farm (San Miguel: 600 pigs, Cebadilla: 200 pigs) and their distance towards the stream (San Miguel: 100 m, Cebadilla: 500 m).

Reactions to the sewage water inflows in terms of species composition: As the diatom flora of the studied streams was recorded for the first time, and no information is available on the natural variation of the abundance of the species, interpretation of the ecological preferences of the diatoms is difficult. Although no strong correlation between physicochemical parameters and species abundance were calculated, the data suggest that species as *Achnanthes praecipua*, *Navicula ruttneri* and *Nitzschia fonticola* preferred unpolluted, well-oxygenated waters, whereas species as *Navicula subminuscula*, *Gomphonema parvulum* and *Nitzschia palea* were stimulated by the sewage waters. *Cocconeis placentula*, a species regarded as sensitive to pollution (Lange-Bertalot 1979), could not be considered as sensitive in this study, as it occurred in high abundance at polluted sites as well as at unpolluted ones, regardless on the pollution level.

These results correspond to results from the Rio Grande de Tárcoles (Silva-Benavides 1996a) concerning the most common species. In both studies, *Gomphonema parvulum*, *Navicula subminuscula*, *Navicula seminulum* and *Nitzschia palea* were common at the polluted sites, and reached at least once a relative abundance of 10% or more. These species are well-known from polluted sites in temperate rivers (Fabri & Leclercq 1984, Krammer & Lange-Bertalot 1986, 1988, 1991a, 1991b, van Dam *et al.* 1994), as well as from other tropical sites affected by pollution (Podzorski 1984, Guerrero & Rodriguez 1991).

However, the species composition at undisturbed sites in this study was largely different from pristine sites in other tropical (Podzorski 1984, Paaby 1988, Silva-Benavides 1996a) and temperate regions (Maier 1988). In the present study *Achnanthes praecipua*, *Navicula ruttneri*, and *Nitzschia fonticola* were common at unpolluted sites. In Río Tárcoles (Costa Rica) Silva-Benavides (1996b) recorded *Achnanthes minutissima*, *Cymbella silesiaca*, *Nitzschia frustulum*, and *Rhoicosphenia abreviata* at undisturbed sites, while Paaby (1988) found *Achnanthes lanceolata* and *Gomphonema angustatum* to be dominant in the Surá stream at La Selva Biological Reserve (Costa Rica). Distinct diatom assemblages in pristine sites can be due to great variations in environmental parameters such as temperature, pH, alkalinity, conductivity, and geology. With increasing organic pollution, parameters like low dissolved oxygen, high biochemical oxygen demand, high concentrations of nutrients as phosphate, nitrate, and ammonia, seem to have an overriding effect on species composition, favoring the development of particular species.

The introduction of sewage waters from the pig farms induced evident changes in the diatom species composition. However, as pointed out by Jongman *et al.* (in Cox 1991) these changes could be also due to other environmental factors like light incidence, water velocity, grazing, and substratum type. Physical, chemical and biological factors are usually interrelated, and it is difficult to separate them. Therefore to get information on the influence of different factors on benthic diatoms and to improve their reliability as bioindicators, experiments under controlled physical and chemical conditions were necessary.

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RESUMEN

Se estudió el efecto de aguas negras de porquerizas sobre la calidad del agua y las comunidades de diatomeas en dos rios tropicales. En las zonas del río adyacentes a la salida de aguas negras se encontró concentraciones elevadas de fosfato, amonio y nitrito. Debido a la gran cantidad de materia orgánica asociada con la actividad bacteriana, se obtuvo un bajo porcentaje de oxígeno. Por medio de la autopurificación del agua, la concentración de amonio bajó y la de nitrato subió en sitios ubicados río abajo de los puntos de descarga de las porquerizas. En total se encontraron 127 especies, 13 de las cuales se conocen sólo de zonas tropicales. La mayoria de ellas fueron encontradas en la naciente del rio. La abundancia de las especies dominantes fue correlacionado con los parámetros físico-químicos de cada lugar y fecha de muestreo. Se observaron las siguientes tendencias: *Nitzschia fonticola* ocurrió sobre todo en sitios limpios; *Gomphonema parvulum*, *Navicula subminuscula* y *Nitzschia palea*, en sitios contaminados; y *Cocconeis placentula* en altas abundancias independientemente del grado de la contaminación. Un análisis de cluster mostró que las comunidades de diatomeas de la naciente se distinguen de los demás sitios. La luz y la corriente del agua pueden ser factores importantes, que influyen las comunidades de la naciente.

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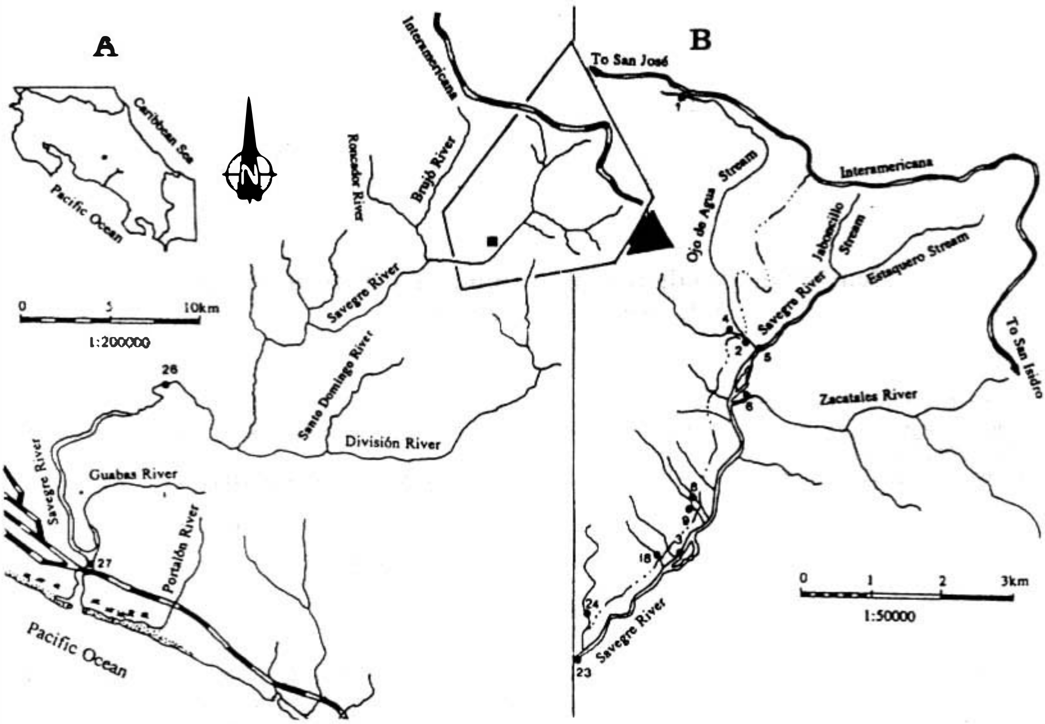

Fig. 1. Rio Savegre basin and sampling sites. (A) Overview of the whole catchment and position of sites SA-26 and SA-27 in the lower part. (B) Detail of upper part of the catchment with location of sites SA-1. SA-2. SA-3. SA-4. SA-5, SA-6, SA-8. SA-9. SA-18. SA-23. SA-24.

cember to April. The river originates in the Cordillera de Talamanca at 3200 m of altitude, draining mainly marine sediments and igneous rock. Due to the negligible human presence in the basin, pollution is insignificant. Therefore low values of nutrients, especially nitrate, were found (Silva 1994). The rather high levels of phosphate and silicate can be explained by the weathering of the igneous bedrock, underlaying the upper marine sediments characteristic for the geology of this area (Weyl 1957). This river and its tributaries are very clear and slightly alkaline. In the uppermost part, Río Savegre is a cool mountain stream, surrounded by dense forest and some pasture land. In the lowest section it is a warm river, mainly surrounded by crop and cattle farms. The chemical and physical features of the river are summarized in Table 1.

Sampling sites: Samples were collected monthly in the period from January 1991 to April 1992 from the main river and several tributaries in the upper part (altitude 2890 - 2300 m a.s.l.) and in the lower part (altitude 200-10 m a.s.l.) of Río Savegre (Fig. 1).

The sampling sites were distributed along an altitudinal gradient between the Ojo de Agua headwaters and the mouth of Río Savegre. Two reaches at SA-1, SA-2, SA-3, SA-5, SA-6, SA-26 (Fig. 1), were selected to observe algal colonies. The observation of algal colonies was accomplished using a viewing box of a metal frame with a glass bottom of 20 x 20 cm square following a line transect method (Blum 1957). Each transect was 20 cm wide, and the algae were observed at 20 cm intervals following a fixed rope across the stream. Microscopical observations were made to identify the genus and species of the macroalgae found in each transect. The taxa were identified using Geitler (1932), Bourrelly (1972,