pothesis that methionine enkephalin has a neurotransmitter role in decapod crustaceans[19,20].

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Temporal organization in population density of protozoans in septic tank sewage

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Variation in the population density of protozoans of a septic tank sewage from a boys' hostel located within the premises of Pt. Ravishankar Shukla University, Raipur, was studied over two consecutive years. Cosinor technique was used to analyse time series data to validate statistically significant annual rhythms in population density of protozoans. Results reveal that rhythmic patterns in population density of various species of sarcodines appear to be highly synchronized with peaks occuring in between mid-March and the first week of July. During the comparable time period at least 6 species of flagellates and 1 species of ciliates showed temporal synchrony with that of the sarcodines. Results of this study may help in optimizing sewage treatment practices involving protozoans.

SEWAGE is a complex ecological system with a rich abundance of organisms ranging from viruses to higher vertebrates. Among all the organisms, bacteria form the bulk and their role in treating sewage has been adequately investigated[1–4]. Apart from bacteria, protozoans also constitute one of the major components of sewage biodiversity. Attempts have also been made to analyse the role of protozoans in the treatment of sewage[5–11]. The sewage from activated sludge plant, oxidation ponds, etc. have been widely studied for various characteristics, but the sewage from septic tanks has been least studied. Furthermore, studies on rhythms in septic tank protozoans are meager.

In this study, therefore, attempts were made to examine temporal organization in the population density of large number of species belonging to three different classes of protozoans in septic tank sewage.

Thirty-six samples were collected over a period of 24 consecutive months at the rate of two equidistant samples per month in the first year and one sample per month in the following year, from a septic tank of a boys' hostel located in the premises of Pt. Ravishankar Shukla University, Raipur. The samples were brought to the laboratory in plastic cans for observing various protozoan types and their density. The types of protozoans were identified using appropriate keys[12–14]. For determining the population density of protozoans, drop count method was employed[15]. One drop of sample (0.05 ml) was placed on a glass slide and covered with a cover glass of 18 × 18 mm size. Protozoans, within the microscopic field were then counted. Simultaneously, the area under the field was measured. This procedure was repeated at several points on the slide. The population density was expressed as number of organisms per ml of

*For correspondence.

the sample after making appropriate conversions for area and volume.

An annual rhythm in population density of protozoans was characterized by the parameters of the best-fitting cosine function approximating all data[16]. The rhythm parameters, estimated by this least square method include the mesor (M, rhythm-adjusted annual mean), the amplitude (A, half of the difference between minimum and maximum of the best-fitting cosine function), the acrophase (ϕ, the time of the annual peak obtained from this cosine function with local midnight of December 31 as the phase reference) and PR (per cent rhythm that shows variability accounted for by the fitted model. A rhythm is detected with regard to the considered period ($\tau = 365.25$ days) when the amplitude differs from zero (non-null amplitude test) with $P < 0.05$.

The results showed that twenty-five species of protozoans which included 4 sarcodines, 7 flagellates, and 14 ciliates, were identified (Table 1). Twelve-month-fitted cosine curves for annual changes in population density of all the species belonging to the class Sarcodina, Flagellata and Ciliata are illustrated in Figures 1 *a–d*. Each curve was obtained by using the mathematical model, $Y_{t_i} = M + A\cos(\omega_{t_i} + \phi)$. Figure 2 documents annual mesor (± 1 SE) for annual variation in population density of the protozoans. The amplitudes of annual variation in population density of each species were expressed as percentage of their respective mesors

Table 1. List of protozoan species identified in the septic tank sewage

Class	Species	Species code
Sarcodina	*Arcella vulgaris*	Av
	Amoeba radiosa	Ar
	Amoeba vespertilio	As
	Amoeba verrucosa	Au
Flagellata	*Thylacomonas compressa*	Th
	Cryptomonas ovata	Cr
	Ancyromonas contorta	An
	Peranema trichophorum	Pr
	Bodo caudatus	Bo
	Oicomonas socialis	Os
	O. termo	Ot
Ciliata	*Paramecium caudata*	Pa
	P. multimicronucleatum	Pu
	Coleps hirtus	Co
	Euplotes patella	Eu
	Stylonychia mytilus	St
	Dileptes anser	Di
	Litonotus fasciola	Li
	Trachelophyllum pusillum	Tr
	Chilodonella uncinata	Ch
	Aspidisca lyncus	Ap
	Colpodo steinii	Cl
	Cledoctema acanthocrypta	Ct
	Vorticella campanula	Va
	V. convallaria	Vo

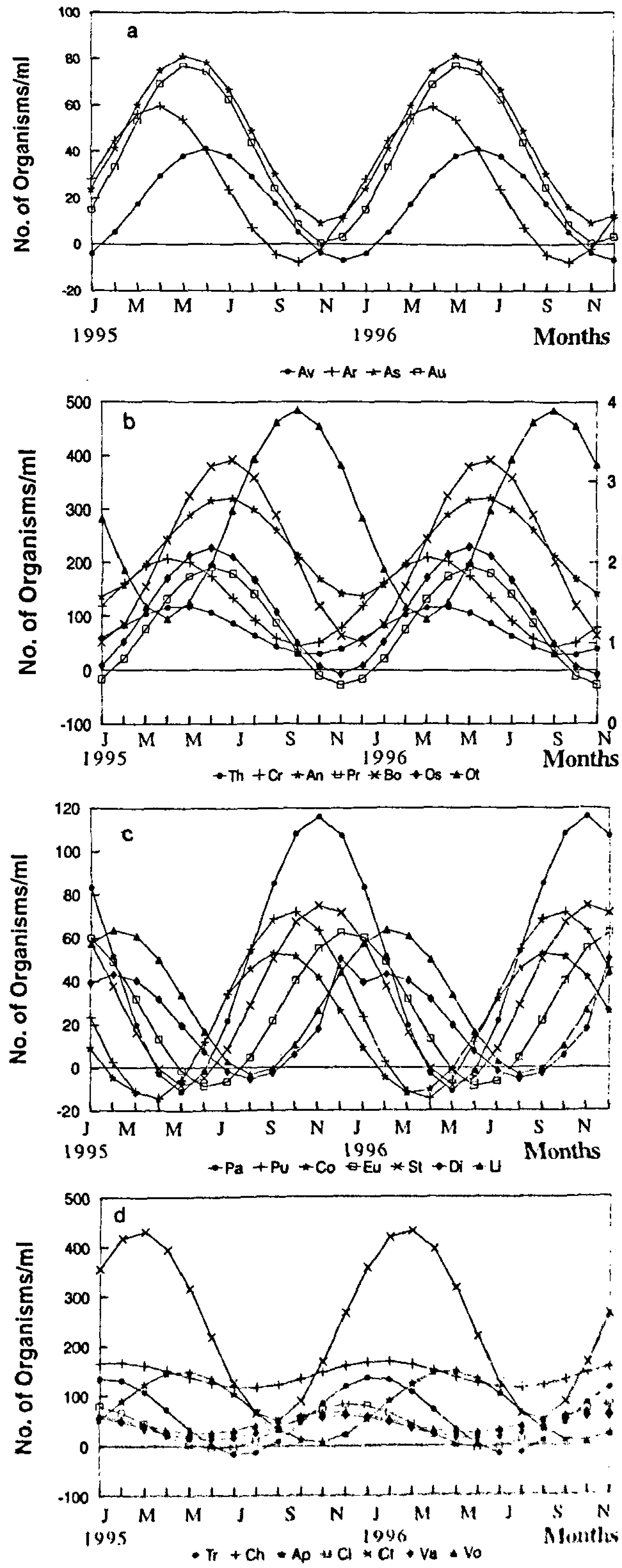

Figure 1. Annual rhythm in population density of various protozoan specie belonging to (*a*) Sarcodina; (*b*) Flagellata, and (*c* and *d*), Ciliata. The dat obtained approximates a 12-month cosine function, where $Y_{t_i} = M + A\cos(\omega_{t_i} + \phi)$. See Table 1 for species code. Population of species A (number × 10^4) and Ot (number × 10^5) are shown in the axis to the right.

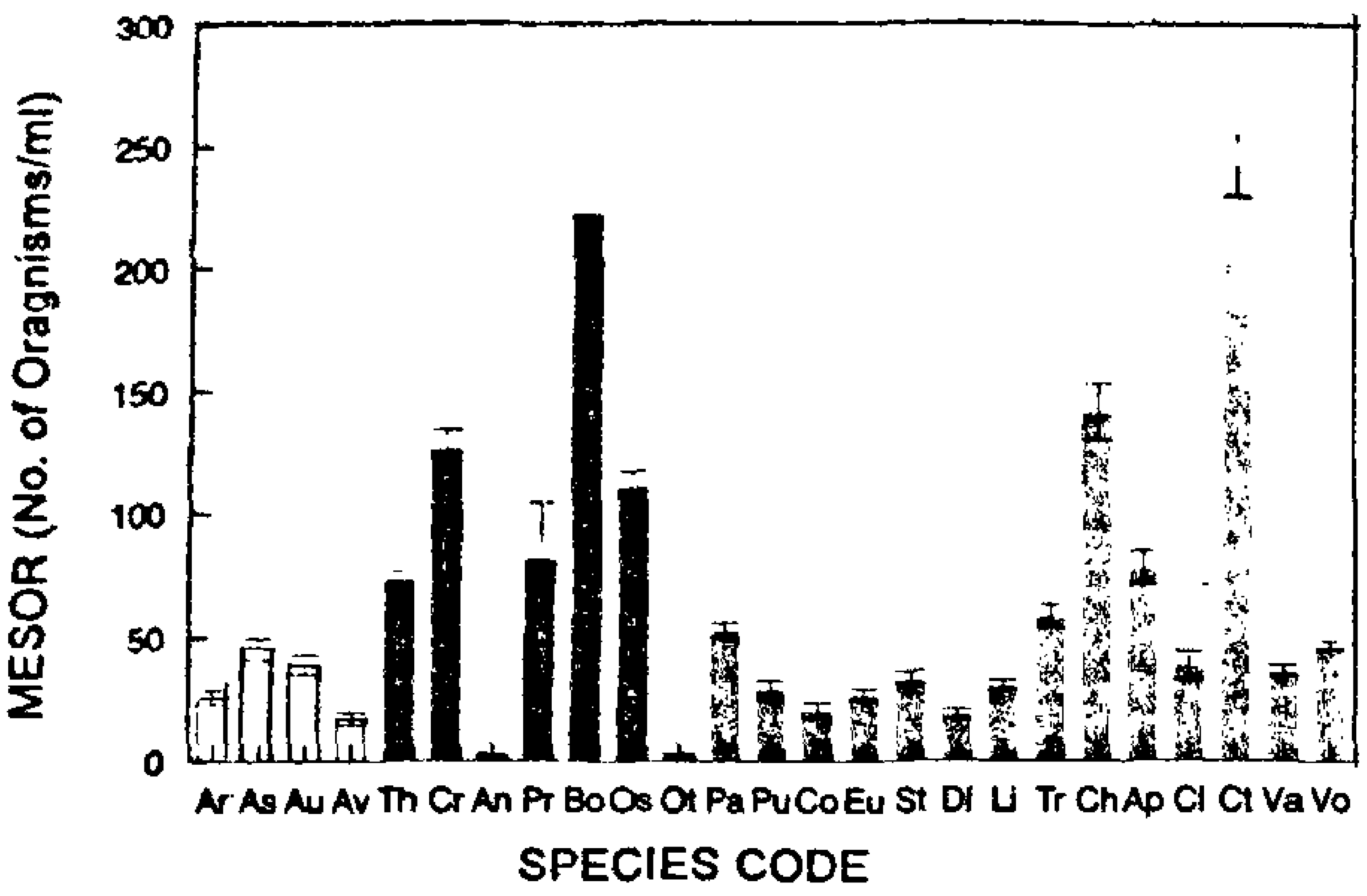

Figure 2. Annual mesor for population density (± 1 SE) of protozoans in septic tank sewage. See Table 1 for species code.

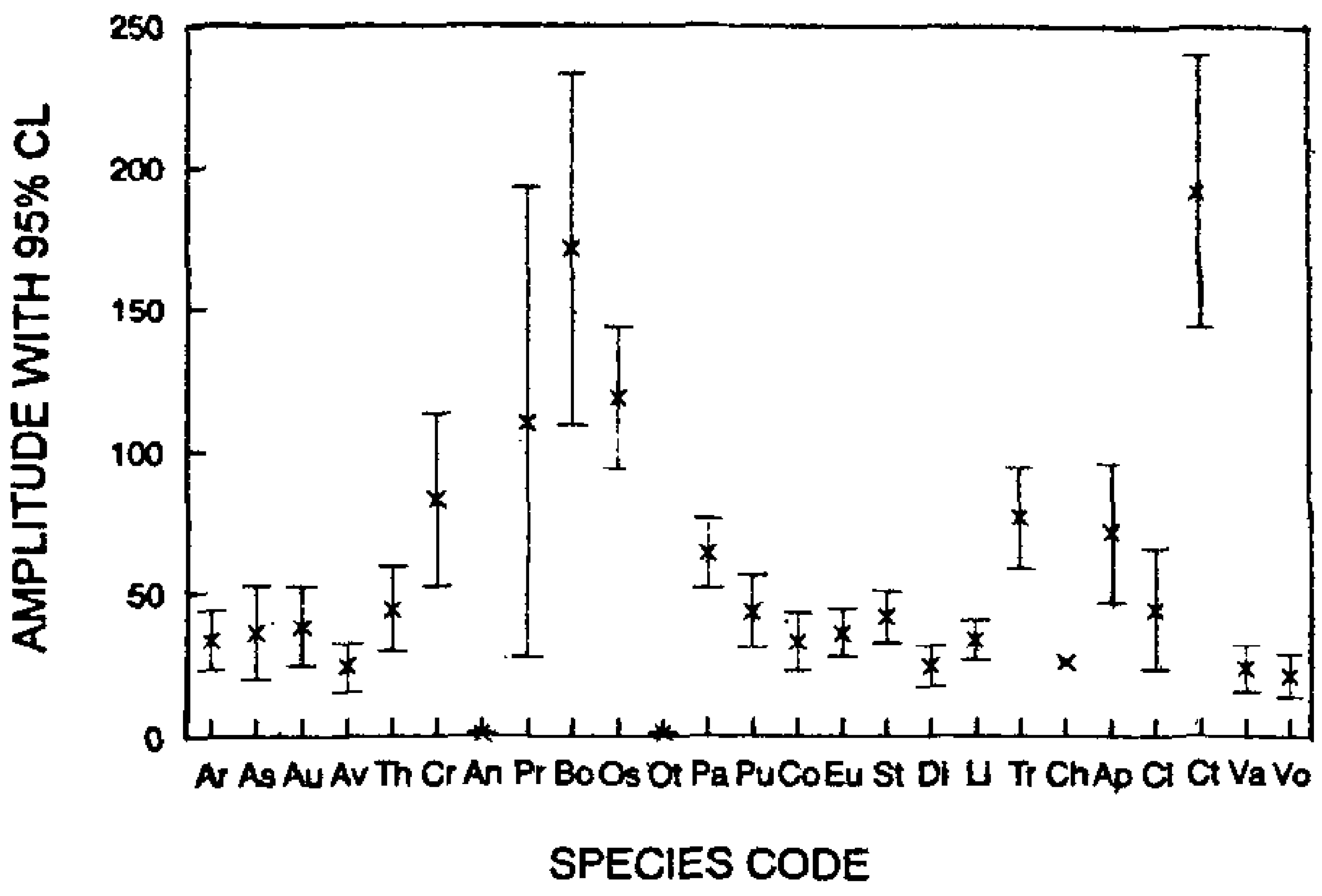

Figure 4. Amplitude map: The cross indicates point estimation for the amplitude in population density in a given species. The vertical line defines 95% CL of the amplitude. See Table 1 for species code.

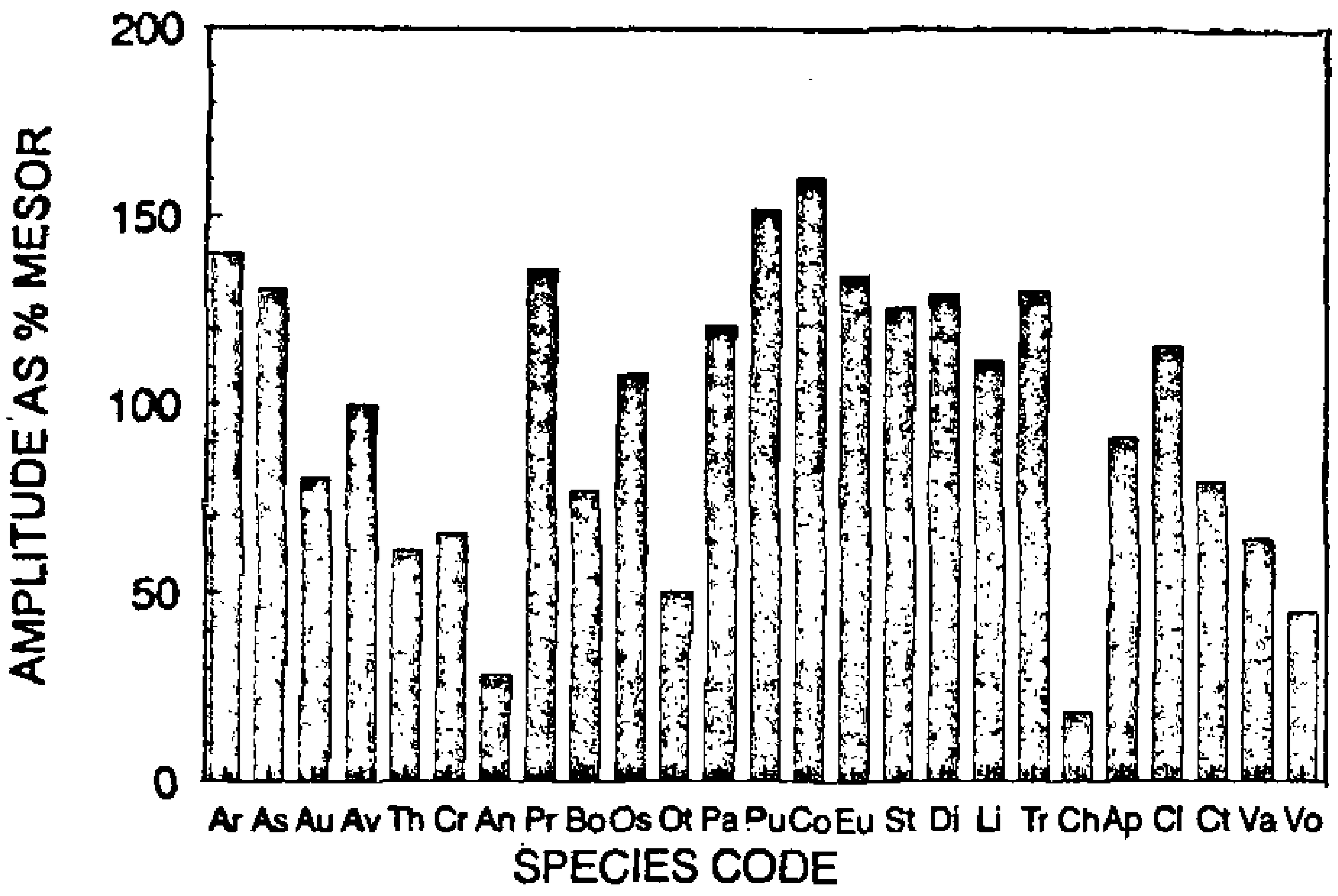

Figure 3. Histogram illustrating amplitudes expressed in % of their respective mesors of annual rhythm in population density of various protozoan species. See Table 1 for species code.

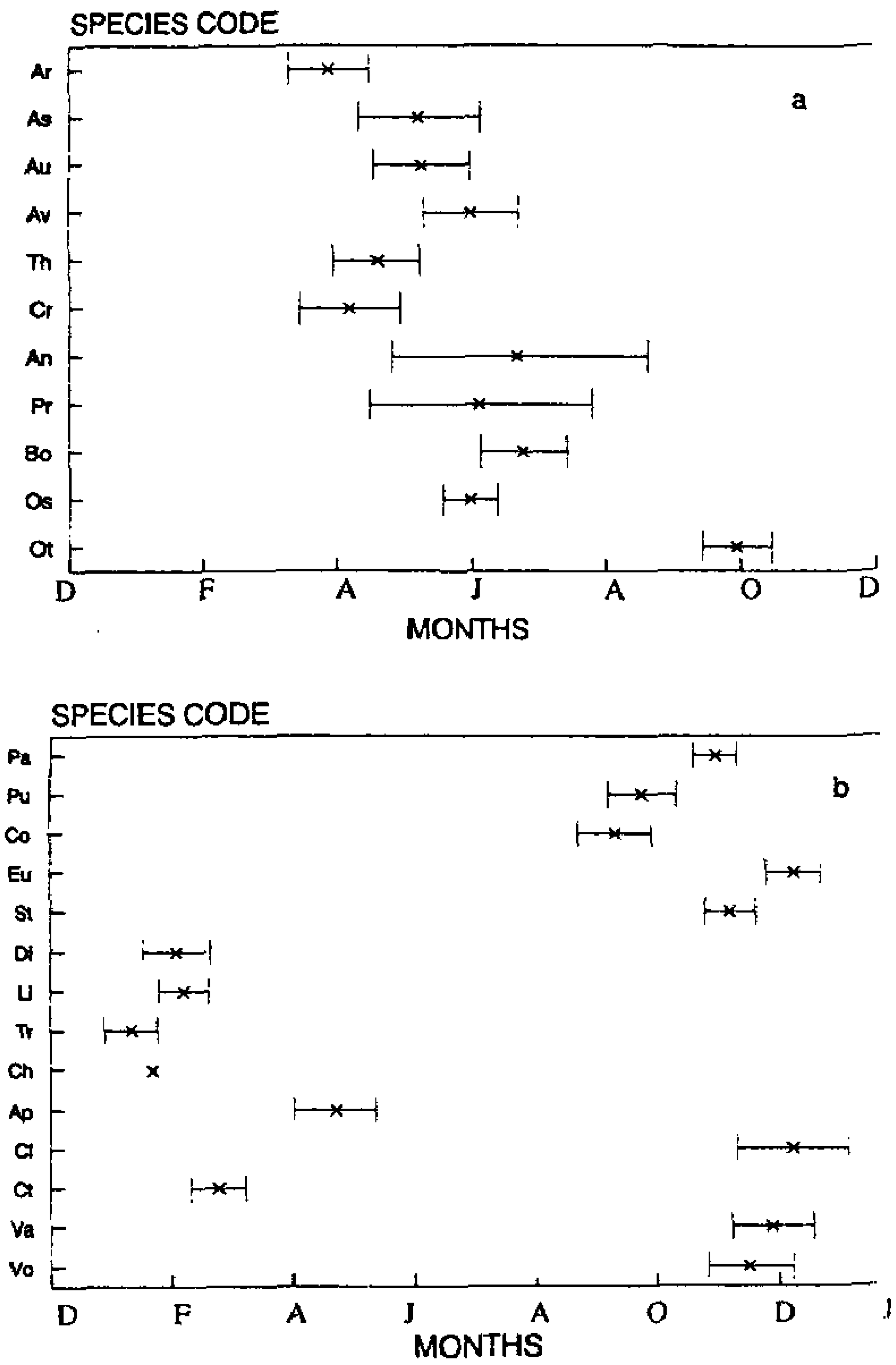

Figure 5. Peak map: (*a*) Sarcodines and Flagellates, and (*b*) Ciliates. The cross indicates point estimation for the peak in population density in a given species. The horizontal line defines 95% CL of the peak. See Table 1 for species code.

(Figure 3). This was done in order to bring uniformity in amplitude of each species. The species, namely (represented in the text by their various species code, see Table 1), Ar, As, Au, Av, Pr, Bo, Os, Pa, Pu, Co, Eu, St, Di, Li, Tr, Ap, Cl, and Ct exhibited very high amplitude variations in their respective annual cycle of population density. Species, such as An and Ch showed very low amplitude variation in their population density. The remaining species, namely Th, Cr, Ot, Va, and Vo showed moderate amplitudes with respect to their mesors. In Figure 4, amplitudes are shown with their respective confidence limits (95% CL). In some species the amplitudes were found to be greater than their respective mesors. This is the reason why in some figures the population density in those species, during certain months, appeared to be either zero or beyond in the negative.

Figure 5 depicts peak map for various species of protozoa with respect to their temporal organization in population density. All species could be categorized into 3 groups. The group 1 species were abundant predominantly between the months of November and March, and the confidence limits of their peaks more or less overlapped with each other. This group includes species, namely Di, Li, Tr, Ch, Pa, Cl, Ct, Eu, St, Va and Vo. The species belonging to the second group were maximally prevalent between mid-March and early-September. The confidence limits of their respective peaks nearly coincided each other. The species belonging to this category are Ar, As, Au, Av, Th, Cr, An, Pr, Bo, Os and Ap. The last group, which included only three species, namely Ot, Co and Pu, was abundant between early September and November. Figure 6 illustrates the percentage of variability accounted for by the fitted model.

Table 2 shows the temporal profile of population density of 25 protozoan species. Table 3 depicts correlation between population density of various protozoan species and physico-chemical parameters. Remarkably, almost all species belonging to Sarcodina showed statistically significant positive correlation both with temperature and total alkalinity of sewage water. In contrast, 57% and 28% species belonging to Ciliata exhibited statistically significant negative correlation with the above physico-chemical variables, respectively. Of the Flagellata, 71% and 43% species showed statistically significant positive correlation with temperature and pH, respectively. Furthermore, 43% species belonging to Flagellata exhibited statistically significant both positive correlation with total alkalinity and negative correlation with dissolved oxygen.

Thus these studies clearly indicate that peak in abundance of protozoans appears to be well organized over an annual time scale and on the basis of their abundance, *vis-à-vis* month of the year, it was possible to classify them into 3 major groups. It seems that one group excludes another or succeeds the other over a linear time scale. Many studies have been conducted to explain spatial succession in different ecosystems. To the best of our information, this is the first study that demonstrates temporal succession among the protozoans inhabiting the domestic sewage. The population density cycle of all the 4 species belonging to the class Sarcodina appears to be highly synchronized, and the peaks in their population density appeared between mid-March and July. During the comparable time period, at least 6 species of flagellates and 1 species of ciliate showed temporal synchrony with the sarcodines. Do they benefit each other? This study is limited in some extent to answer the above question, nevertheless it would be worthwhile to examine the community relationship between the species, i.e., those which show temporally synchronous generation cycle. Similar phenomena for

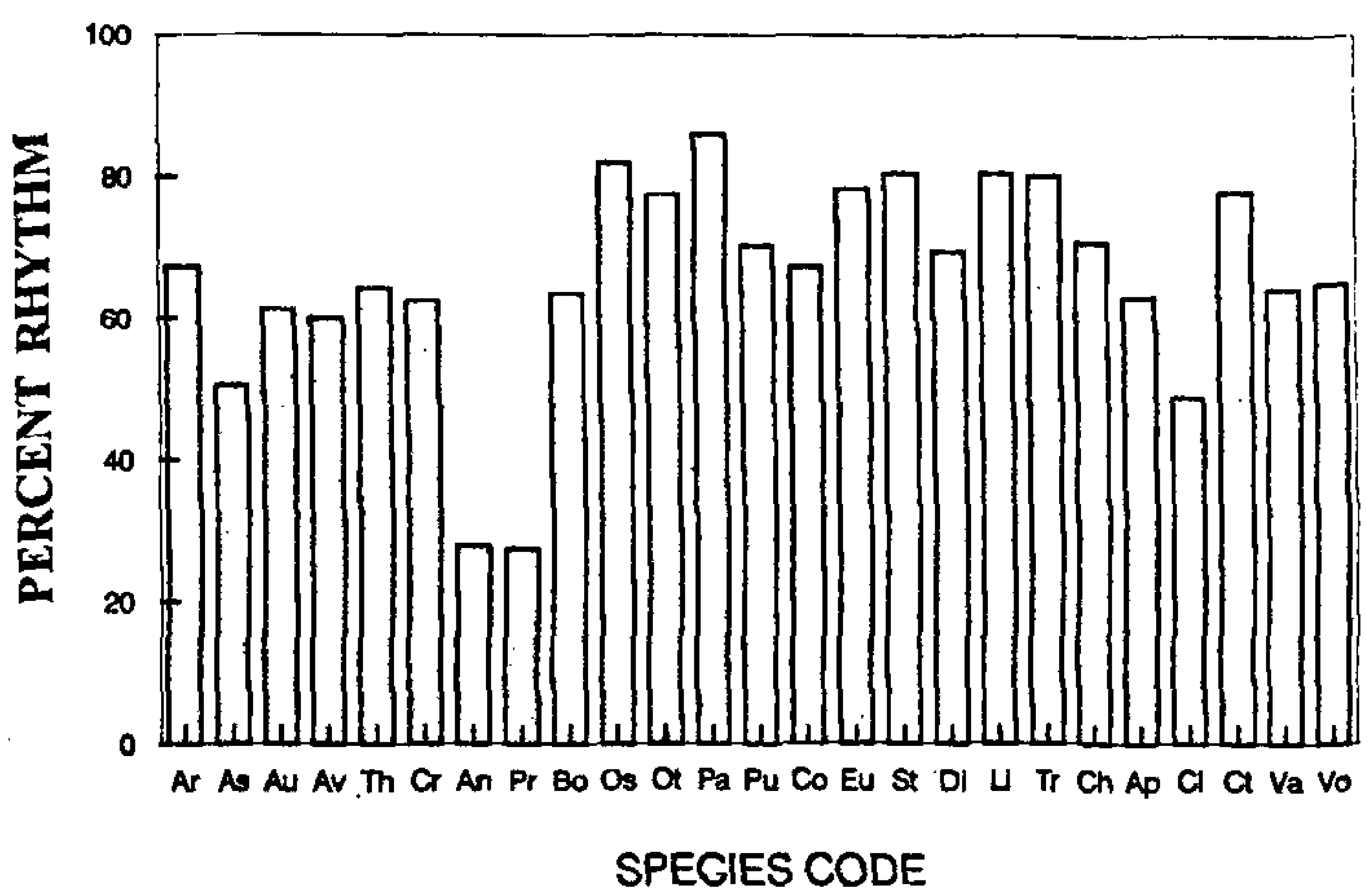

Figure 6. Histogram illustrating per cent rhythm for the annual rhythm in population density of various protozoan species (see Table 1 for species code). PR denotes the percentage of variability accounted for by the fitted cosinor model.

other two groups (first and third) which were predominantly abundant between November and March and September and November, respectively, largely remain unexplained. Further, the temporal profile of various species of protozoans was complemented by data on correlation coefficient obtained from their population density with respect to the prevailing time-qualified physico-chemical status. All the species which showed their abundance between mid-March and the first week of July invariably exhibited statistically significant positive correlation with both temperature and total alkalinity. In contrast, most of the ciliates peaking between November and March showed statistically significant negative correlation with temperature, pH, and total alkalinity. However, it seems to be mandatory to perform some experiments to substantiate the above. Furthermore, the relative significance of temporal abundance of these species with respect to other nonprotozoan microorganisms in sewage has not been investigated in this study.

There may be many more factors that can optimize the growth or population of a given species like the volatile solids and several elements, including the heavy metals[17,18]. To this may be included the biological parameters, for example, grazing by rotifers, nematodes, other parasites and viruses[19–21].

Protozoan population density changes with respect to factors like volatile solids which are used as food, and some of the elements act as essential nutrients[22]. Some of the heavy metals, like Ca, Cu etc., are required in larger or smaller quantities as essential elements[23]. Some of the elements present in sewage, like Cr, are nonessential. Essential or nonessential, heavy metals in larger quantities are always harmful and exert inhibitory effect on protozoans population[23]. Similarly, viruses and

Table 2. Temporal profile of population density of 25 protozoan species

Month		Ar	As	Au	Av	Th	Cr	An	Pr	Bo	Os	Ot	Pa	Pu	Co	Eu	St	Di	Li	Tr	Ch	Ap	Cl	Ct	Va	Vo
1995																										
July	a	25*	47	73	30	86	110	1.9	200	400	250	2.8	0	0	0	0	0	0	0	0	80	113	0	0	7	20
	b	20	1	25	15	66	106	2.1	800	500	282	2.9	33	47	40	0	5	50	0	0	80	47	0	0	20	20
Aug.	a	7	10	10	13	40	66	2.4	400	300	245	3.5	53	60	40	4	13	0	0	0	80	27	0	0	27	30
	b	0	27	15	15	35	80	2.6	20	300	190	3.5	70	70	53	8	25	0	0	0	133	20	0	0	50	40
Sep.	a	0	47	30	15	30	90	3.4	10	400	105	3.9	73	90	73	10	47	0	0	0	180	13	40	100	40	50
	b	0	73	40	13	65	95	3.4	7	400	100	4.2	90	100	93	20	100	0	0	33	170	10	50	120	50	60
Oct.	a	0	53	33	46	7	140	3.1	0	400	65	4.1	113	110	75	27	87.	0	0	47	180	7	73	133	47	60
	b	0	27	27	0	50	120	2.1	0	240	40	4.4	113	110	53	33	80	0	20	53	180	10	80	160	60	90
Nov.	a	0	10	13	0	52	105	1.7	0	140	10	4.0	126	80	35	50	73	10	27	60	187	30	80	210	67	80
	b	0	0	0	0	46	70	1.9	0	95	0	3.1	140	50	15	80	80	10	33	73	233	33	140	253	80	70
Dec.	a	0	0	0	0	32	32	1.2	0	0	0	2.8	120	27	0	80	87	20	30	87	290	40	160	270	90	67
	b	0	0	0	0	40	25	1.1	0	0	0	2.5	100	10	0	100	73	27	40	107	193	50	90	283	60	60
Jan.	a	13	0	0	0	42	40	0.7	0	0	0	2.4	67	0	0	80	67	30	40	107	120	60	93	305	50	47
	b	53	27	0	0	66	66	0.7	0	0	15	2.2	47	0	0	50	60	30	50	107	160	80	53	330	40	40
Feb.	a	60	47	20	0	75	146	0.9	10	0	45	2.1	40	0	0	50	53	40	60	180	180	80	47	440	40	60
	b	73	53	47	0	106	252	2.3	11	100	50	1.6	30	0	0	40	33	40	60	200	220	87	40	462	33	50
Mar.	a	87	60	53	0	140	252	2.4	60	300	115	1.4	7	0	0	27	8	47	67	167	233	100	27	470	33	40
	b	93	80	60	7	145	266	2.9	110	340	140	1.4	0	0	0	20	0	50	70	120	180	127	13	490	30	33
Apr.	a	73	87	80	33	155	252	3.0	160	400	150	1.4	0	0	0	7	0	50	70	100	160	140	0	470	33	30
	b	60	106	100	40	146	220	3.4	190	200	145	1.0	0	0	0	0	0	40	60	60	120	187	0	390	27	40
May	a	47	110	120	55	126	192	3.6	130	340	155	1.2	0	0	0	0	0	30	50	0	120	220	0	360	27	40
	b	45	120	120	80	120	180	2.8	140	352	240	1.3	0	0	0	0	0	20	40	0	100	280	0	365	20	33
June	a	33	90	80	80	100	180	2.4	130	300	246	1.7	0	0	0	0	0	10	30	0	100	160	0	260	13	27
	b	25	87	80	52	100	115	2.1	120	300	235	2.2	0	0	0	0	0	7	20	0	90	127	0	120	7	10
1996																										
Sep.	a	0	80	60	30	33	70	3.0	0	200	80	4.0	60	50	80	20	40	0	0	–	90	60	0	80	20	30
Oct.	b	0	40	20	10	40	70	–	0	120	53	4.4	80	55	60	20	70	0	7	40	80	0	20	100	20	50
Nov.	a	0	0	0	0	40	0	–	0	100	0	3.3	120	60	40	25	45	20	30	106	40	0	20	120	45	67
Dec.	a	0	0	0	0	27	0	–	0	–	0	–	147	70	40	47	47	45	60	120	20	20	40	160	60	60
Jan.	a	0	0	0	0	20	–	–	0	–	0	–	120	20	0	80	60	70	60	128	10	45	67	160	65	70
Feb.	a	10	0	0	0	40	–	–	0	36	0	3.0	40	0	0	53	20	60	80	120	–	60	80	–	45	50
Mar.	a	25	–	–	0	53	–	1.5	–	150	120	2.8	35	0	0	20	20	20	60	80	–	60	60	–	36	–
Apr.	a	33	–	–	7	67	200	–	–	200	288	–	20	0	0	0	5	0	20	40	–	80	120	–	–	–
May	b	60	80	40	20	120	200	–	40	200	200	–	–	0	0	0	0	0	0	0	220	93	–	280	–	–
June	a	67	40	60	40	127	173	2.0	67	300	147	1.6	–	0	0	0	0	0	0	0	180	110	–	300	20	40
July	b	20	40	32	40	80	100	2.0	80	–	120	1.5	–	0	20	0	0	0	0	–	120	120	0	160	10	33
Aug.	a	0	40	30	20	67	80	–	32	–	123	–	40	20	20	10	0	0	0	–	80	120	0	100	7	–

*Number of organisms/ml, except the values for An (number × 10^4) and Ot (number × 10^5); a, first week of the month; b, third week of the month; See also Table 1 for species code.

Table 3. Correlation between population density of the protozoans and physico-chemical characteristics

Species code[a]	Physico-chemical parameters: Temperature	pH	Dissolved oxygen	Total alkalinity
Sarcodina				
Av	0.72[b],*** (34)[c]	0.30 (34)	−0.20 (34)	0.45** (34)
Ar	0.44** (34)	0.17 (34)	0.11 (34)	0.40* (34)
As	0.80*** (32)	0.42* (32)	−0.17 (32)	0.62*** (32)
Au	0.80*** (32)	0.44** (32)	−0.24 (32)	0.70*** (32)
Flagellata				
Th	0.68*** (34)	0.36* (34)	−0.07 (34)	0.53*** (34)
Cr	0.63*** (31)	0.26 (31)	−0.20 (31)	0.48** (31)
An	0.71*** (26)	0.63*** (26)	−0.58** (26)	0.51** (26)
Pr	0.28 (32)	0.23 (32)	−0.25 (32)	0.13 (32)
Bo	0.68*** (30)	0.48** (30)	−0.56*** (30)	0.35 (30)
Os	0.74*** (34)	0.19 (34)	−0.33* (34)	0.24 (34)
Ot	−0.18 (28)	−0.22 (28)	−0.32 (28)	−0.46* (28)
Ciliata				
Pa	−0.66*** (31)	−0.12 (31)	−0.01 (31)	−0.42* (31)
Pu	−0.19 (34)	0.02 (34)	−0.30 (34)	−0.30 (34)
Co	−0.04 (34)	0.08 (34)	−0.33* (34)	−0.23 (34)
Eu	−0.83*** (34)	−0.41* (34)	0.38* (34)	−0.36* (34)
St	−0.68*** (34)	−0.36* (34)	0.08 (34)	−0.47** (34)
Di	−0.33* (34)	0.05 (34)	0.31 (34)	0.30 (34)
Li	−0.27 (34)	−0.02 (34)	0.32 (34)	0.36* (34)
Tr	−0.60*** (31)	−0.21 (31)	0.60*** (31)	−0.06 (31)
Ch	−0.04 (31)	−0.22 (31)	0.05 (31)	−0.16 (31)
Ap	0.60*** (34)	0.28 (34)	0.01 (34)	0.65*** (34)
Cl	−0.60*** (32)	−0.54** (32)	0.09 (32)	−0.41* (32)
Ct	0.08 (31)	−0.01 (31)	0.30 (31)	0.38* (31)
Va	−0.68*** (32)	−0.28 (32)	0.04 (32)	−0.27 (32)
Vo	−0.56*** (30)	−0.14 (30)	0.03 (30)	−0.23 (30)

[a]Refer Table 1 for details; [b]Correlation coefficient; [c]degrees of freedom; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Range for physico-chemical parameters; temperature, 22°C–34°C, pH, 7.2–7.9; DO, 0.0–0.8 mg/l; total alkalinity, 180–586 mg/l.

grazers have also inhibitory effects, but availability of those organisms which act as food source, for example bacteria, do exert stimulatory effects on the population size of protozoans[1,24,25].

Sewage treatment is the most important procedure that often encounters serious problems[4]. These problems could be classified under two major heads: biological and nonbiological. It is well known that bacteria constitute the major bulk of the population of microorganisms of the domestic sewage[1]. Therefore, most often investigators try to manipulate population of various species of bacteria in order to treat sewage[1]. Surprisingly, the protozoans are neglected, although they constitute the bulk of microbial population next to bacteria, in sewage. Concludingly, the study documents annual rhythm in protozoan community in septic tank sewage. Certain species seem to synchronize their rhythms. On the basis of that, three groups appear to emerge and this phenomenon may be attributed to the influence of factors such as temperature. Results of this study led us to recommend that the abundant forms of ciliates of the septic tank recorded here could be tested further in order to achieve one of the major goals: sewage treatment. This study becomes particularly important in light of the inferential statistics which has been used to predict the time of abundance of various protozoan species in domestic sewage. Thus, these results may help in optimizing sewage treatment practices involving protozoans.

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Natural occurrence of monoploids and polyploids in the Indian catfish, *Heteropneustes fossilis*

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Natural occurrence of male and female haploid, triploid and tetraploid *Heteropneustes fossilis* is reported for the first time. Karyotypic and nuclear volumetric evidences are described to confirm the haploid, triploid and tetraploid nature of the identified individuals. Studies on spermatogenesis also confirm the observed unusual ploidies in these individuals.

OWING to the absence of well-defined sex chromosomes in fishes, polyploidy has spontaneously originated, perhaps repeatedly, and has been sustained in populations of diverse orders[1]. In fact much has been written on this subject by Ohno[2]. Expectedly, natural triploid populations have evolved in 8 genera representing 3 orders of fish; the viviparous Poeciliids (*Poecilia, Poecilopsis*)[2], oviparous Cyprinids (*Carassius*[4]; *Misgurnus*[5]; *Phoximus*[6]; *Rutilus*[7]) and Athernids (*Menidia*)[8]. Viable hybrid unisexual triploids have also been recorded in *Poecilia*[9]: (i) *P. latipinna-2 mexicana*, and (ii) *P. latipinna-mexicana*; *Poecilopsis*[10]: (i) *P. 2 monacha-lucida* (ii) *P. monacha-2 lucida*, and (iii) *P. monacha-viriosa-lucida*; and *Phoximus*[11]: (i) *P. 2 eos-neogaeus*, and (ii) *P. eos- 2 neogaeus*[1]. Likewise, naturally occurring tetraploids have been reported in *Carassius auratus*[4], the European *Barbus* sp.[12], *Misgurnus anguillicaudatus*[5] and *Cobitis biwae*[13]. The frequency with which unusual base replacements, inactivating mutations can occur in the duplicated genes, and the workload for replication has perhaps minimized the abundance of tetraploid fish species[14].

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In India, Pandey and Lakra[15] recorded tetraploidy in a single individual of *Clarias batrachus*, using karyotype ($2n = 50$; $4n = 100$) as the evidence. While listing chromosome number of several fishes, Manna[16] doubted the possible occurrence of polyploidy in *Heteropneustes fossilis*, but provided no supporting evidence for it. Tiwary *et al.*[17] claimed successful induction of triploidy in *H. fossilis*, but have not provided acceptable evidence for their claim. To the best of our knowledge, no report is as yet available for natural occurrence of haploids (male and female) in any fish species, although the publication of Varadaraj[18] stands out as a single publication on the induction of viable haploid gynogenetic *Oreochromis mossambicus*.

This present communication reports on the natural occurrence of monoploids and polyploids (triploids and tetraploids), both in males and females in the South Indian populations of the catfish, *H. fossilis*, on the basis of the evidences of erythrocyte nuclear volume and karyotype. We have also shown the unconventional mechanism of spermatogenesis in these naturally occurring polyploids.

Collections of *H. fossilis* were made from different sources in Tamilnadu and Kerala during April–October 1998. As many as 120 individuals were randomly selected from these populations, for confirmation of their ploidy groups. Initially, a number of individuals were sacrificed to acquire blood for erythrocyte nuclear measurement[19], and tissues for karyotyping[20]. However, all subsequent analyses were made following noninvasive procedures, as live monoploids and polyploids were required for further studies: Hence, blood was collected by caudal puncturing. Smears were fixed in methanol for 1 min and stained in 4% Giemsa in phosphate buffer (pH 6.4) for 10 min, and were subjected to nuclear measurements using stage and ocular micrometer (Erica, Japan) · under a phase contrast microscope (Nikon, Japan).

Table 1 shows the occurrence of monoploids, diploids, triploids, and tetraploids at the frequency of 1.7, 91.7, 4.2 and 2.5% respectively. In all these unusual ploidy groups, both males and females were recorded, though the frequency of female triploid and tetraploid was only one each, against 4 and 2 males, respectively.

Ploidy was identified on the basis of both erythrocyte (RBC) volume, and chromosome number. The nuclear volume of RBC increased from 4.1 μm^3 in a haploid to 8.7, 13.7 and 19.5 μm^3 in diploid, triploid and tetraploid, respectively (Figure 1). In tetraploids, the nuclear volume widely varied, compared to other ploidy groups.

The diploid chromosome number varied between 56 and 58. Of the 110 individuals analysed, as many as 86 individuals had 58 chromosomes, 15 individuals had 56, and the rest 57. Of the 120 individuals analysed, 2 proved to be haploids, of which the female bore 30