Jpn. J. Phycol. (Sôrui) **36**: 17–23, March 10, 1988

Seasonal change of planktonic protista collected from Shioya Coast, Osaka Bay

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HARA, S. and TAKAHASHI, E. 1988. Seasonal change of planktonic protista collected from Shioya Coast, Osaka Bay. Jpn. J. Phycol. **36**: 17–23.

Diatoms were numerically the most dominant protist group among the autotrophic protista. Dinoflagellates, cryptophytes and prasinophytes were the subdominant groups. Cell numbers of the autotrophic protista were generally small during the seasons of falling and low water temperatures (from October to March), while large numbers were recorded during the seasons of rising and high water temperatures (from April to September). Among the heterotrophic protista, choanoflagellates and apochlorotic chrysophytes were numerically dominant. Apochlorotic cryptophytes, ebridians and apochlorotic dinoflagellates were the subdominant groups. The numerical relationship between the autotrophic and heterotrophic protista is represented by a regression line described by the equation: $H=1.62\ A^{0.553}$, where A is the number of the autotrophic cells and H is the number of heterotrophic cells.

Key Index Words: autotrophic protista—cell number—heterotrophic protista—Osaka Bay—seasonal change.

It is well known that autotrophic protista of nanoplanktonic dimensions are the major contributors to cell number, cell volume, amount of chlorophyll pigments and primary productivity in the planktonic community in the sea (SAIJO 1964, PARSONS 1972, BOOTH *et al.* 1982, FURUYA and MARUMO 1983). Relatively large members of the heterotrophic protista include herbivorous species and they may be the major contributors in the grazing food chain (SIEBURTH 1979, FENCHEL 1980). By contrast, many of the relatively small members are thought to feed on bacteria and detritus particles of bacterial size (SIEBURTH 1979, DAVIS and SIEBURTH 1984). These bacteriophagous protista may connect bacteria to animals of higher trophic levels in a detritus food chain in the sea: the pathway is dissolved organic matter-bacteria-bacteriophagotrophic protista-animals of higher levels (HARA and TANOUE 1984, TANOUE and HARA 1986). A qualitative as well as quantitative estimation of these small organisms must be taken into consideration for an accurate assessment of the actual condition of the marine ecosystem. The present study has focussed mainly on an elucidation of the cell number, composition and change of the heterotrophic as well as the autotrophic protista.

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture (59540421).

Materials and methods

Samples of surface seawater were collected at the end of a jetty (about 10 m long) of Shioya Coast (Osaka Bay) in polyethylene bottles (Fig. 1). The sampling was conducted at monthly intervals from May 1979 to December 1980. Temperature of the surface seawater was measured by a mercury

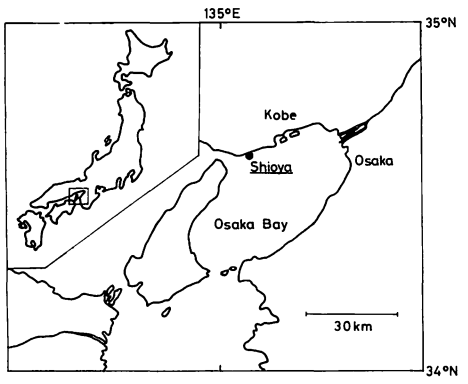

Fig. 1. The location of the sampling point (Shioya Coast) in Osaka Bay.

thermometer. The protist samples in the seawater were fractionated with a plankton net (NXXX25 with 40 μm mesh aperture). Protist samples which passed through the net were examined. The filtered sample (total volume 1,000 m*l*) was divided into two equal portions. One portion was fixed with 1% Lugol solution, allowed to settle in a glass bottle for a week, and concentrated to a volume of 0.5 to 5 m*l*. The cell number of each species of protista in concentrated sample was counted under light microscope with a Thoma hemocytometer. The other portion of the sample was concentrated by centrifugation (1,400×g, 10 min.) and examined in two ways: (1) by direct observation of living material by light microscopy and (2) by electron microscopic observation of the sample prepared by whole mount method. Scale-bearing protista (e.g. those belonging to chrysophytes, prymnesiophytes, prasinophytes, Centrohelida in heliozoans), diatoms and choanoflagellates were identified and counted by electron microscopy. The cell number of each protist (N), group counted by electron microscopy, was transformed into the number of cells in a unit volume of seawater (n) by the following formula:

$$n = N \times D_l / D_e,$$

where D_e is the number of diatom frustules enumerated by electron microscopy, and D_l is the total number of diatom frustules with and without protoplast in a unit volume of seawater examined by light microscopy. In electron microscopical examination, a JEM-100B analytical electron microscope was used.

Results

Autotrophic protista

Autotrophic protista are defined as those unicellular eucaryotes that have chlorophyll pigments in their plastids. The concentration of the autotrophic protista ranged from 5 cells/m*l* on 16 December , 1980 to 57,000 cells/m*l* on 13 August, 1980 and the average concentration was 9,000 cells/m*l* (Fig. 2b). No typical spring bloom of the autotrophic protista was detected in Shioya Coast. Cell numbers were quite different from one sample to the other. In order to characterize the pattern of seasonal change of the autotrophic protista, a year was divided into four seasons, i.e. low (January to March), rising (April to June), high (July to September) and falling (October to December) water temperature seasons (cf. Fig. 2a). The mean water temperatures of these four seasons were 9.5°C, 17.1°C, 24.2°C and 18.3°C respectively.

Large cell numbers of the autotrophic protista (larger than 10,000 cells/m*l*) were obtained in four samples (July, 1979 and April, August and September, 1980) collected during the seasons of rising or high water temperatures (Figs. 2a, b). Small cell numbers (smaller than 1,000 cells/m*l*) were obtained in eight samples (November and December, 1979, and February, March, May, July, October and December, 1980). Six of these eight samples were collected during the seasons of falling or low water temperatures, and the other two (May and July in 1980) were obserbed during the seasons of rising and high water temperatures.

Diatoms were the most numerous and the commonest group of the autotrophic protista in Shioya Coast. They were found throughout the investigation period. In ten

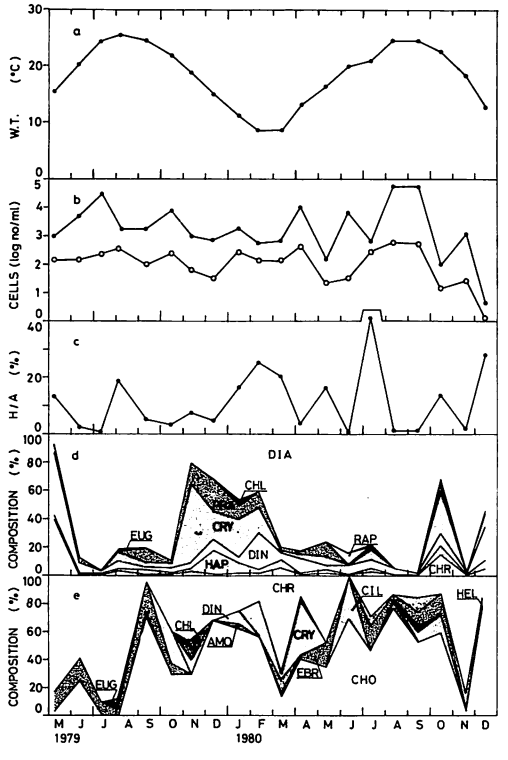

Fig. 2. Seasonal changes of the surface seawater temperature and numerical parameters of protista in Shioya Coast from May, 1979 to December, 1980. (a) The surface seawater temperature. (b) Cell numbers of the autotrophic (solid circle) and the heterotrophic (open circle) protista. (c) Ratio of the heterotrophic protist cells to the autotrophic protist cells (%). (d) Taxonomic composition of the autotrophic protista. (e) Taxonomic composition of the heterotrophic protista. Abbreviations are as follows; CHL, chlorophytes; PRA, prasinophytes; EUG, euglenophytes; CRY, cryptophytes; DIN, dinoflagellates; DIA, diatoms; PRY, prymnesiophytes; RAP, raphidophytes; CHR, chrysophytes; EBR, ebridians; CHO, choanoflagellates; AMO, amoebae; HEL, heliozoans; CIL, ciliates.

of the eleven samples collected during rising and high water temperature seasons (including the two samples of small cell numbers), diatoms occupied more than 75% of the total cell number (Figs. 2a, d). In contrast, in five of the nine samples obtained during falling and low water temperature seasons, diatoms occupied only half or smaller part of the cell number of the autotrophic protista. In all of these diatom-poor samples the cell numbers were smaller than 1,000 cells/m*l*. A year was divided into two preiods by the dominancy of diatoms; (1) rising and high water temperature seasons when diatoms were dominant, and (2) falling and low water temperature seasons when diatoms were poor (Fig. 2d). *Skeletonema* was the dominant genus in diatoms; and dinoflagellates, cryptophytes and prasinophytes were the subdominant groups. Dinoflagellates, which were observed throughout the investigation period, occupied more than 20% of the autotrophic protista in May, 1979 and February and December, 1980 (Fig. 2d); *Gymnodinium* and *Glenodinium* being the dominant genera. The cryptophytes occupied more than 20% of the autotrophic protista in falling and low water temperature seasons (Fig. 2d), *Cryptomonas* being the dominant genus. Prasinophytes occurred constantly in cell number throughout the investigation period, which occupied more than 10% of the autotrophic protista from December, 1979 to February, 1980 (Fig. 2d). *Pyramimonas* was the dominant genus in prasinophytes. Chrysophytes, prymnesiophytes, raphidophytes, euglenophytes and chlorophytes constituted a rather smaller fraction of the autotrophic protista (Fig. 2d). *Kephyrion* was the dominant genus in the chrysophytes and was distributed widely in every season. Prymnesiophytes, dominated by the genus *Chrysochromulina*, occurred throughout the investigation period (Fig. 2d). A dense population of *Heterosigma* of the raphidophytes was found in June, 1980. Sometimes small cell numbers of euglenophytes and/or chlorophytes occurred in the protist samples (Fig. 2d). The dominant genera in these groups were *Eutreptia* and *Chlamydomonas*, respectively.

The autotrophic protista removed by filtration contained a small number of large diatoms, such as *Coscinodiscus, Licmophora,* and large dinoflagellates, such as *Ceratium.*

Heterotrophic protista

Heterotrophic protista are the eucaryotes without chlorophyll pigments. They in-

clude the apochlorotic forms of phytoprotista as well as protozoa.

The cell number of the heterotrophic protista ranged from 1.3 cells/m*l* (December, 1980) to 610 cells/m*l* (August, 1980), with an average of 190 cells/m*l*. The seasonal change in the cell number of this group corresponded to that of the autotrophic protista (Fig. 2b), and large cell numbers occurred during high water temperature season, about 600 cells/m*l* in August and September in 1980. The cell number of the heterotrophic protista was 11% of the cell number of the autotrophic protista in average, and was largest (41%) in July, 1980.

Marine species of the heterotrophic protista obtained in this study were classified into ten groups (Fig. 2e), six belonging to apochlorotic phytoprotista (chlorophytes, euglenophytes, cryptophytes, dinoflagellates, ebridians and chrysophytes), and four to protozoa (choanoflagellates, amoebae, heliozoans and ciliates). Choanoflagellates and apochlorotic chrysophytes were the two major groups of the heterotrophic protista in Shioya Coast (Fig. 2e). In nine of the twenty samples collected, choanoflagellates occupied more than 50% of the total cell numbers of the heterotrophic protista (Fig. 2e). *Diaphanoeca* and *Acanthocorbis* were the two dominant genera in choanoflagellates. In six of the twenty samples collected, apochlorotic chrysophytes occupied more than 50% of the total cell number of heterotrophic protista (Fig. 2e), *Calycomonas* and *Paraphysomonas* being the two dominant genera. During rising and high water temperature seasons, ebridians occurred constantly and sometimes abundantly (Fig. 2e). In the heterotrophic cryptophytes, *Chilomonas* was found abundantly in November, 1979 and from February to April, 1980 (Fig. 2e). Sometimes apochlorotic dinoflagellates occurred abundantly, occupying 24.1% of the total cell number of the heterotrophic protista in October, 1979 (Fig. 2e). Apochlorotic *Gymnodinium* was the dominant genus in the heterotrophic dinoflagellates. Ciliates, heliozoans, amoebae and apochlorotic euglenophytes and chlorophytes were the minor constituents of the heterotrophic protista in Shioya Coast. The dominant genera in ciliates were *Helicostomella*; in heliozoans, *Heterophyrs* and *Acanthocystis*; in euglenophytes, *Dinema* and in chlorophytes, *Polytoma*. Amoeboid forms were not identified.

The heterotrophic protista removed by filtration were dominated by *Noctiluca* and ciliates. Ciliates occurred in May and August, 1979 and January and April, 1980, when large number of the heterotrophic protista was recorded.

Discussion

Generally every member of the autotrophic as well as heterotrophic protista, with some exception in minor members, occurred in Shioya Coast throughout the investigation period (Figs 2d, e). There was an obvious seasonal fluctuation in the composition of the autotrophic protista, while there was no obvious pattern of seasonal fluctuation in the composition of the heterotrophic protista (Fig. 2e). From rising to falling temperature seasons, it was diatoms, dinoflagellates and euglenophytes; while from falling to low temperature seasons, it was cryptophytes that formed higher percentages of the autotrophic protista (Fig. 2d). In the heterotrophic protista, ebridians were abundant in rising and high water temperature seasons, while apochlorotic cryptophytes were abundant in falling and low water temperature seasons. However, the two major groups of the heterotrophic protista, choanoflagellates and apochlorotic chrysophytes, showed no obvious seasonal fluctuation in Shioya Coast (Fig. 2e).

Choanoflagellates were the dominant heterotrophic protista not only in coastal waters (CHRETIENNOT 1974, this investigation) but also in oceanic habitats, such as the Bay of Alaska (BOOTH *et al.* 1982) and the Southern Ocean (BUCK and GARRISON

1983, HARA *et al.* 1986). Choanoflagellate is one of the most important heterotrophic protista in the surface sea water. Apochlorotic chrysophytes have been reported as a common member of protista in the study of the flora of coastal waters examined by electron microscope (THRONDSEN 1969, LEADBEATER 1974, MOESTRUP 1979, TAKAHASHI 1987). Quantitative seasonal change of apochlorotic chrysophytes has not been reported to date. The present study revealed that apochlorotic chrysophytes occurred numerously throughout the investigation period, being one of the dominant heterotrophic protista in Shioya Coast. This suggests that apochlorotic chrysophytes are one of the dominant as well as the common members of the heterotrophic protista in the coastal waters of the world.

The numerical relationship between the cell numbers of the autotrophic and heterotrophic protista is illustrated in Figure 3. Although the population of the heterotrophic protista investigated in this study includes bacteriophagous as well as phytophagous species, their existence basically depends upon the primary production of the autotrophic protista. It is obvious that the seasonal change of the cell numbers of the heterotrophic protista is related to that of the autotrophic protista (Fig. 2b). These two groups showed a linear correlation on a logarithmic scale (Fig. 3a). The regression line is represented by the equation:

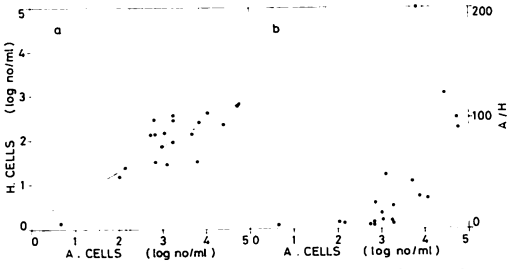

Fig. 3. Relationship between the cell numbers of autotrophic (A) and heterotrophic (H) protista. (a) Linear correlation between the autotrophic and the heterotrophic protist cell numbers. The regression line is: $H=1.62\ A^{0.553}$ (r=0.819). (b) The ratio of autotrophic cells to heterotrophic cells (A/H) plotted against the logarithm of autotrophic cells per m*l*.

$$H = 1.62\ A^{0.553},\ (r=0.819)$$

where A is the number of the autotrophic protist cells and H is the cell number of the heterotrophic protista. The exponent (0.553), which is smaller than one, indicates that the larger the cell number of the autotrophic protista, the larger is the ratio of the autotrophic protist cells to the heterotrophic protist cells (A/H) (Fig. 3b). It also indicates that the 10-fold change of the autotrophic cells causes only 3.6-fold change of the heterotrophic cells. It suggests that the fluctuation in the cell number of the heterotrophic protista is smaller than that of the autotrophic protista. The smaller fluctuation in the cell number of the heterotrophic protista is in agreement with the results obtained in the case of protist population in the surface water of the Southern Ocean (HARA and TANOUE 1985, HARA *et al.* 1986). Since Osaka Bay and the Southern Ocean are geographically isolated and their environmental conditions are quite different, the components of the autotrophic and the heterotrophic protista were not the same in these two localities (HARA and TANOUE 1985, HARA *et al.* 1986), but the small variability of heterotrophic protista may be a common phenomenon in both localities.

In the present study, the concentration of protista which passed through the net (NXXX25) were estimated as 9,000 cells/m*l* in average (5–57,000 cells/m*l*) for the autotrophic protista and 190 cells/m*l* in average (1–610 cells/m*l*) for the heterotrophic protista. Averaged values of the cell numbers of nanoprotista (smaller than 20 μm in size) in the coastal waters of the western North Atlantic were 3,100 cells/m*l* (700–6,400 cells/m*l*) for the autotrophic and 3,000 cells/m*l* (900–5,000 cells/m*l*) for the heterotrophic protista (DAVIS and SIEBURTH 1982). There is an obvious difference in the cell numbers (both averages and ranges) between the heterotrophic protista in Shioya Coast and those in the western North Atlantic. In addition to the regional

difference, the difference of heterotrophic cell number may also be attributable to the difference in the methods of enumeration in each estimation. The cell number of heterotrophic protista was estimated with a hemocytometer coupled with an electron microscope in the present study. There is a possibility that colorless heterotrophic organisms were overlooked, therefore the cell number of heterotrophic protista in this study may be an underestimation. DAVIS and SIEBURTH (1982) calculated the cell number of heterotrophic protista by subtracting the number of cells with emission of chlorophyll pigments from the total number of protist cells counted by acridine orange staining epifluorescence microscopy. Because of the inability to observe the cellular detail with epifluorescence microscopy, the organisms identified as heterotrophic protista by their method may include not only zoospores of foraminifera (DAVIS and SIEBURTH 1982) but also autotrophic protista with quenched fluorescence of their chloroplasts. The cell number of heterotrophic protista measured by their method may be an overestimation. In addition to their epifluorescence microscopical method, morphological observations of the cells with transmission light and electron microscopics are essential for accurate estimation of the cell number and the composition of the protist community.

Many of the heterotrophic protista are known to be bacterial feeders (LAVAL 1971, LEADBEATER and MORTON 1974, SIEBURTH 1979, SIEBURTH and DAVIS 1982, DAVIS and SIEBURTH 1984). These heterotrophic protista may be capable of consuming bacteria over a wide range of concentrations found in both coastal and oceanic waters (DAVIS and SIEBURTH, 1984). These protista can be eaten effectively by larger animals, such as Antarctic krills, in the Southern Ocean (HARA and TANOUE 1984, TANOUE and HARA 1986). Hence, heterotrophic protista such as choanoflagellates, apochlorotic chrysophytes and dinoflagellates and amoebae may be the major consumers of the bacterial biomass in marine habitats. They may connect the bacterial biomass to the animals of higher trophic levels in the detritus food chain in marine ecosystems.

Acknowledgement

The authors thank Dr. S.A. SALEHI of Osaka University for reading the manuscript.

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原　成光*・高橋永治：大阪湾塩屋海岸における原生生物群集の季節的変動**

独立栄養原生生物では珪藻が多産した。次いで渦鞭毛藻，クリプト藻，プラシノ藻が多かった。珪藻細胞数は，水温下降期から低水温期（10月－3月）にかけては少なく，水温上昇期から高水温期（4月－9月）にかけては多かった。従属栄養原生生物群では，襟鞭毛虫と無色の黄金色藻が多産した。次いで無色のクリプト藻，エブリア類，無色の渦鞭毛藻が多かった。独立・従属両栄養生物群の細胞数は次式の関係にあった：$H = 1.62A^{0.553}$，ここでAは独立栄養原生生物群の，Hは従属栄養原生生物群の細胞数を表わす。（*657 神戸市灘区六甲台　神戸大学大学院自然科学研究科，**657 神戸市灘区六甲台　神戸大学理学部）