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Infraspecific differences in *Cyclotella comta* populations in the Fuji Five Lakes and Lake Ashino-ko in Japan

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The valve diameter (D), marginal width (M) and striae (S) and cell densities of *Cyclotella comta* populations together with the nutrient profile of the water were examined by using samples collected from coastal surface water at thirty-five stations in the Fuji Five Lakes and Lake Ashino-ko in 1987. The natural populations of *C. comta* were found to show a statistically significant annual change in the valve diameter and markings on the valve surface. In a population, M decreases, but S increases and M/D does not change or increases, when D decreases, while the correlation coefficients of D–M, D–S and D–M/D decreases in the order. D, M and M/D get smaller and S larger from spring to autumn, while keeping at the above feature. The cell type defined by two variables such as D–M, D–S or D–M/D seems to be present in each lake. The dependency of cell type on cell density and environmental factors was observed.

Key Index Words: annual cell cycle—niche—Ashino-ko—cell density—cell size distribution—cell type— Cyclotella comta*—diatom—Fuji-goko—nutrient.*

ROBINSON and WALLER (1966) showed geographically separated populations of the marine planktonic diatom, *Rhizosolenia styliformis*. Minor variants recorded in the planktonic diatom *Tabellaria flocculosa* of lakes (ROUND 1965, ROUND and BROOK 1959, KOPPEN 1975) seem to be stable (ROUND 1981). At least in small lake, the surface diatom population is uniform horizontally (MARUYAMA 1988). In the case of man-made lakes such as Lakes Sagami-ko and Tsukui-ko, the cell size distribution of poulation changed continuously with location (NAMIKI *et al.* 1985). In lakes connected by a river such as the Nishina Three Lakes, the differences in cell size among lakes are slightly larger than those within each lake (MARUYAMA 1988). The present study focussed on the differences in form (or shape) appeared and area occupied by infraspecific variants in *C. comta*.

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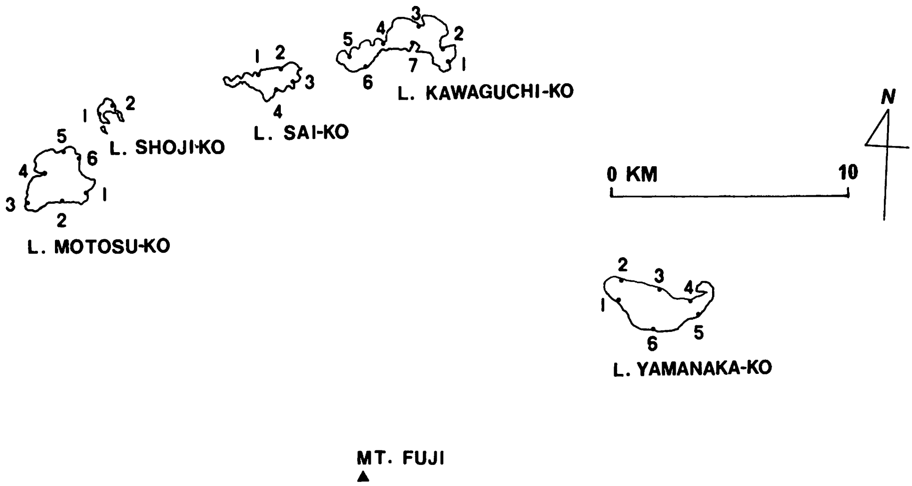
Six lakes from northwest to southeast around Mt. Fuji stand within 50 km. One old lake was divided into three lakes of Motosu-ko, Shoji-ko and Sai-ko by a coulee in 864. Lakes Yamanaka-ko and Kawaguchi-ko were also formed by a coulee in 800 (NOBUHARA *et al.* 1971). Lake Ashino-ko is a crater lake formed in about 1100BC (IGOU 1980). These lakes are spring-fed lakes with very little inflow of water from ground surface (TANAKA 1900, TANAKA *et al.* 1982, YAGI 1974). The details of the Fuji Five Lakes and Lake Ashino-ko (SUZUKI 1982) are shown in Fig. 1.

Materials and Methods

Thirty-five sampling stations were chosen along the shore in six lakes, *viz.* 6 in Lake Motosu-ko, 2 in Lake Shoji-ko, 4 in Lake Sai-ko, 7 in Lake Kawaguchi-ko, 6 in Lake Yamanaka-ko and 10 in Lake Ashino-ko (Fig. 1). Samplings were made on April 12–14,



Lakes	Longitude (E)	Latitude (N)	Altitude (m)	Area (km^2)	Maximum depth (m)
Motosu-ko	138°35'	35°28'	900.0	4.78	121.6
Shoji-ko	138°37'	35°29'	900.0	0.51	15.2
Sai-ko	138°41'	35°30'	900.0	2.15	71.7
Kawaguchi-ko	138°45'	35°31'	830.5	5.60	14.6
Yamanaka-ko	138°52'	35°25'	980.5	6.66	13.3
Ashino-ko	139°00'	35°13'	724.5	7.04	40.6

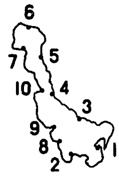


Fig. 1. Sampling stations and the locations, areas and maximum depths of the Fuji Five Lakes and Lake Ashino-ko.

June 14–16, August 9–10, October 14–15 and December 16, 1987. At each station, triplicate surface water samples were collected in 250 m*l* polyethylene bottles; one was fixed in 4% formalin (final concentration) for morphological examination and the other two were used for chemical analyses. The pH and electric conductivity (EC) were measured using pocket glass electrode pH (YEW model PH51) and EC (YEW model SC51) meters.

Water samples of 250 m*l* each were centrifuged at 2,750 × g for 15 min, the sediments resuspended in 2.5 m*l*, and 0.05 m*l* suspensions placed on a cover-glass and dried over a hot-plate at 100°C. Cells were examined by using a microscope (Nikon model Optiphot NTF) and a micro-stage (Sapporo Breweries Ltd. model MS) connected to a computer (Fujitsu model FM-8). Population estimates were based on triplicate cell counts and expressed as number of cells per m*l*. The valve diameter (D), the marginal width (M), and the number of striae in 10 μm of the valve round (S) were measured in 200 cells in each population by using a video-writer (FOR.A model FVW 300) connected to a computer (Fujitsu model FM-11 and FM R-50FD) and M/D was calculated as M × 100/(D/2).

Total nitrogen (T-N) and total phosphorus (T-P) were analyzed by the methods of JAPANESE STANDARDS ASSOCIATION (1986), and silica (SiO_2) and COD by the methods of

NIHONSUIDOKYOUKAI (1985).

Results

Division into populations with different reproductive cycles

Each population from Lakes Yamanaka-ko and Ashino-ko in spring is assumed to consist of small cells (S) and large cells (L) with different reproductive cycles (NAMIKI *et al.* 1985). The population in Lake Yamanaka-ko was divided into two groups by a rough estimation at 13.5 μm in diameter and that in Lake Ashino-ko at 12.0 μm (Fig. 2), and the populations obtained were statistically analyzed.

Differences within each lake

Since the distributions of D, M and S of each population were different from each other in form and location, the following procedure was adopted: the judgement whether the distribution is normal (1) or not (2) by the test for detecting the departure from normality (Kolmogorov-Smirnov one sample test, KOT) (Table 1); if it is normal, then the judgement is done whether it is equivariant (3) or not (4) by the test for homogeneity of variance (Bartlet's test, BT) (Table 2). In a judgement on the distribution of D, when the distribution was normal and equivariant [(1) → (3)], (1) → (3) was observed every season in Lakes Motosu-ko and Shoji-ko, (1) → (4) or (2) with the season in Lake Saiko, and (1) → (3) or (1) → (4) with the season in Lakes Yamanaka-ko and Ashino-ko. The distributions of M and S can be stated mostly normal (Table 1).

As some of the lakes at some seasons showed the case (2) or (1) → (4), Kolmogorov-Smirnov two sample test (KTT) was

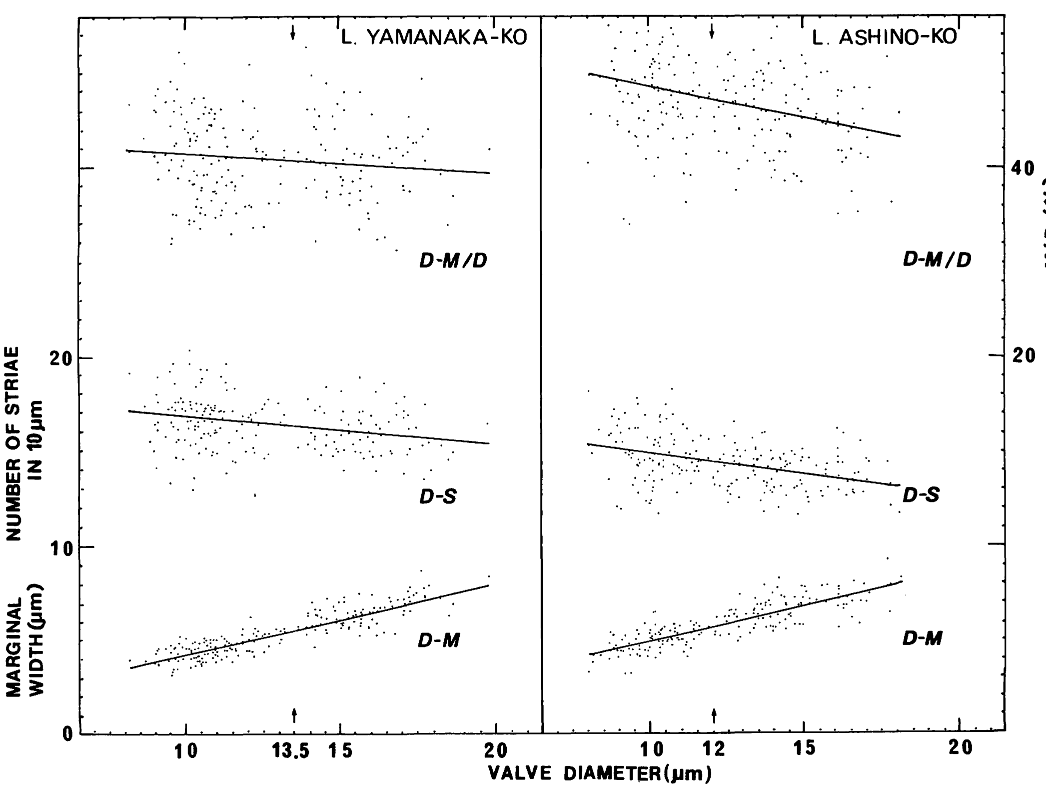


Fig. 2. Relationship of valve diameter (D) to marginal width (M), number of striae (S), and M/D ratio of *C. comta* at station 1 in Lake Yamanaka-ko and station 3 in Lake Ashino-ko in spring in 1987 as examples, indicating small and large populations divided by 13.5 μm in D in Lake Yamanaka-ko and by 12.0 μm in Lake Ashino-ko.

Table 1. The observed largest differences of Kolmogorov-Smirnov one sample test (KOT) of D, M and S in the Fuji Five Lakes and Lake Ashino-ko in 1987. The differences beyond the value for 5 per cent significance are tabulated.

Lakes & Stations		Apr.	June	Aug.	Oct.
Diameter (D)					
L. Sai-ko	1		0.1385		
	3		0.1169		
	4		0.1126		
Number of striae (S)					
L. Motosu-ko	5				0.1154
L. Sai-ko	1				0.1119
	3				0.1192
L. Yamanaka-ko	1		0.0973		
L. Ashino-ko	9		0.1073		
Width of marginal region (M/D)					
L. Motosu-ko	6	0.0979			
L. Ashino-ko	4				0.0983

employed for the detection of differences in population within each lake. The results obtained in many instances with the exceptions indicated by box in Table 3 can be reasonably regarded as having the same distribution at 5 per cent level of significance.

Fig. 3A shows the point and interval estimates of population medians of D, S and M/D. They are also within a narrow range at the same season in each lake. The interval estimates in every other month do not generally overlap with each other and the changes over the lapse of time can be followed. It is seen from the figure that differences in the range and width of D, S and M/D of the annual changes are found among 6 lakes. As one or both of the variables follow a normal distribution, the population was analyzed by the bivariate analysis to check its character.

General character of a population

Regression analysis was used for the evaluation of D–M, D–S, and D–M/D in a population. The regression line can be represented by an equation in the form of $y=\alpha+\beta x$. The equation of the line can be rewritten as $y=\gamma+\beta(x-\bar{x})$. This change is achieved by writing $\gamma=\alpha+\beta\bar{x}$, that is γ is the y-value of the point on the line corresponding to $x=\bar{x}$. In a population, M decreases, S increases and M/D increases or does not change as D decreases. This relation is shown in Fig. 4, in which the point and interval estimates of the regression coefficients β for D–M, D–S and D–M/D are distributed in plus range, mostly in minus range and mostly in minus range with large deviation, respectively, while the correlation coefficients for D–M, D–S and D–M/D decrease in the order (Table 4). This feature seems to be basically common in a population in all seasons and lakes.

Change observed from spring to autumn

D, M and M/D get smaller and S larger as

Table 2. The observed largest differences of Bartlett' test (BT) of D at 5 per cent significance applied to the population having the value of KOT below 5 per cent significance.

Lakes & Seasons	Observed values	5% significances (Degrees of freedom)
L. Motosu-ko		
Apr.	7.77	11.07 (5)
June	7.81	11.07 (5)
Aug.	10.15	11.07 (5)
Oct.	10.31	11.07 (5)
L. Shoji-ko		
Apr	0.46	3.84 (1)
June	0.16	3.84 (1)
L. Sai-ko		
Oct.	8.59	7.81 (3)
L. Yamanaka-ko		
Apr. S*	9.65	11.07 (5)
L*	29.79	11.07 (5)
June	19.64	9.49 (4)
Aug.	14.30	7.82 (3)
L. Ashino-ko		
Apr. S*	21.36	14.07 (7)
L*	9.80	14.07 (7)
June	24.62	14.07 (7)
Oct.	11.01	11.07 (5)

* S, small cell populations; L, large cell populations.

Table 3. The observed largest differences of Kolmogorov-Smirnov two-sample test (KTT) of D in all combinations of stations in each lake at the same season in the Fuji Five Lakes and Lake Ashino-ko in 1987. The values over 5 per cent level of significance, 0.136, are indicated by box.

L. Motosu-ko

	2	3	4	5	6
1	**0.220**	0.110	0.130	0.125	0.115
2		0.135	**0.165**	0.120	**0.210**
3			0.085	0.060	0.125
4 (APR)				0.075	0.095
5					0.110
1	0.115	0.115	0.065	0.085	0.080
2		0.055	0.100	0.050	0.055
3			0.110	0.065	0.065
4 (JUN)				0.075	0.080
5					0.055
1	0.075	0.070	0.090	0.070	0.065
2		0.055	0.070	0.060	0.065
3			0.075	0.075	0.060
4 (AUG)				0.040	0.070
5					0.055
1	0.055	0.050	0.135	0.130	0.095
2		0.060	**0.145**	**0.140**	0.110
3			0.130	0.130	0.070
4 (OCT)				0.080	0.090
5					0.075

L. Shoji-ko

	2
1	0.055 (APR)
1	0.115 (JUN)

L. Sai-ko

	2	3	4
1	0.060	0.130	0.060
2		0.095	0.055
3 (JUN)			0.100
1	0.105	**0.170**	**0.185**
2		0.125	**0.170**
3 (OCT)			0.085

L. Yamanaka-ko

	2	3	4	5	6
1	**0.190**	**0.155**	0.075	0.130	**0.205**
2		0.125	**0.230**	**0.190**	0.110
3			**0.170**	0.110	0.075
4 (APR)				0.100	**0.205**
5					0.130
1	0.100		0.130	0.135	0.075
2			0.110	0.100	0.085
4 (JUN)				0.130	0.060
5					0.110
1	0.115	**0.150**	0.125		
2		0.070	**0.190**		
3 (AUG)			**0.215**		

L. Ashino-ko

	2	3	4	5	6	7	8	9
1	0.090	**0.170**	0.115	0.110	0.080	0.160	0.075	
2		**0.175**	0.130	0.080	0.090	0.150	0.070	
3			0.085	0.120	0.125	0.045	0.125	
4				0.065	0.065	0.090	0.100	
5					0.070	0.105	0.065	
6 (APR)						0.120	0.055	
7							0.115	
1	0.100	0.070	0.065	0.075	0.095	**0.145**		**0.140**
2		0.075	0.070	0.055	0.070	**0.155**		0.115
3			0.060	0.070	0.090	**0.175**		**0.145**
4				0.065	0.085	**0.150**		0.110
5					0.075	0.135		0.125
6 (JUN)						0.125		0.130
7								**0.140**
1	0.065	0.055	0.055	0.120	0.065			
2		0.085	0.050	**0.140**	0.085			
3			0.075	0.095	0.060			
4 (OCT)				0.135	0.060			
5					**0.140**			

the season proceeds, while keeping a population at the above feature. These changes are illustrated with the means in the right portion of Fig. 4A, C–E and the point and interval estimates of γ corresponding to each mean are also shown in Fig. 4. Those in every other month do not mostly overlap with each other. The path swept out by the seasonal changes of means is described as the downward trends of D–M and D–M/D and the upward trend of D–S. With these changes (as D decreases) β of D–M decreases toward zero, that of D–S decreases from zero and that of D–M/D does not change (Fig. 4A, C–E). This annual change also seems to be common to all the lakes.

Differrences in population among lekes

The cell types (see Discussion) characterized by D, M and S seem to be endemic to the lake. This is illustrated as the shift of the paths of D–M, D–S and D–M/D in the right portion of Fig. 4A. The paths of D–M and D–M/D in Lakes Shoji-ko (June), Yamanaka-ko, Shoji-ko (Apr.), Ashino-ko, Motosu-ko, and Sai-ko shift to the left and upward in the order and that of D–S to the left and downward mostly in the order. The interval estimates of γ do not almost overlap with each other with a few exceptions.

The populations in Lake Shoji-ko in spring (group I), those in Lakes Shoji-ko in early summer and Sai-ko (group II), and those in the other lakes (group III) are distinguished from each other by the morphological appearance as shown in Fig. 5. Groups I, II and III are morphologically separable from each other: Group I is separable from other two groups in having high striae density,

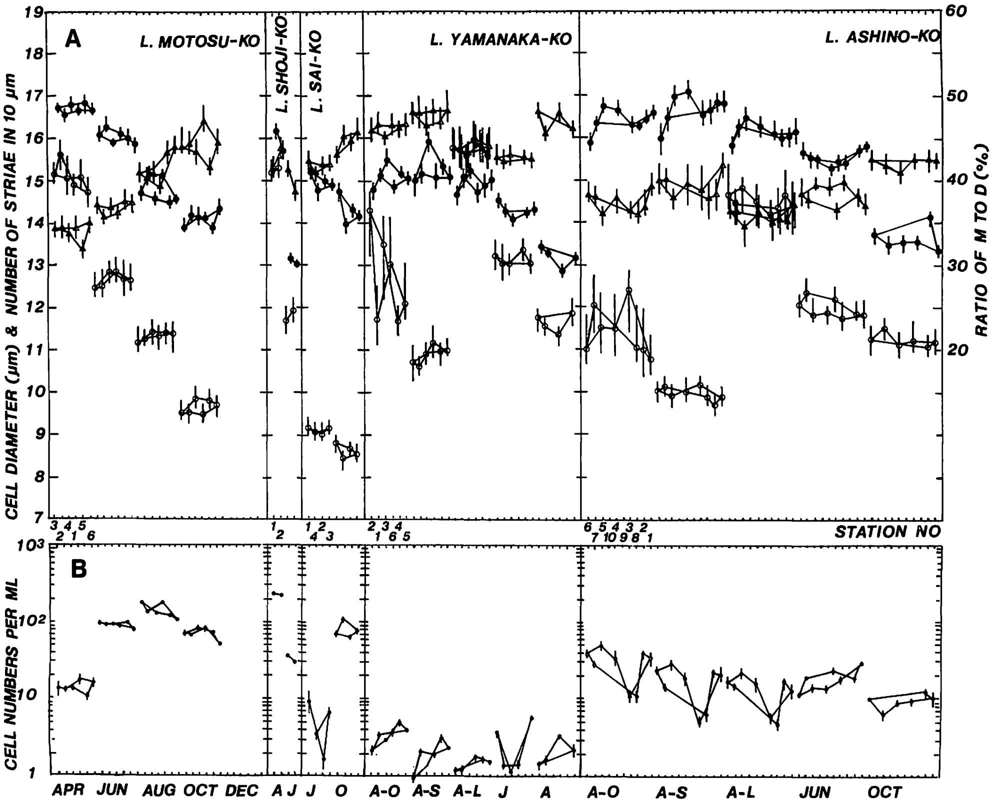


Fig. 3. The seasonal and regional changes of the point and 95 per cent interval estimates of population medians of value diameter (open circle), number of striae (open triangle) and the ratio of marginal width to valve diameter (solids circle) (A), and the population density (B) in the Fuji Five Lakes and Lake Ashino-ko in 1987. O, original population; L, large cell populations; S, small cell populations.

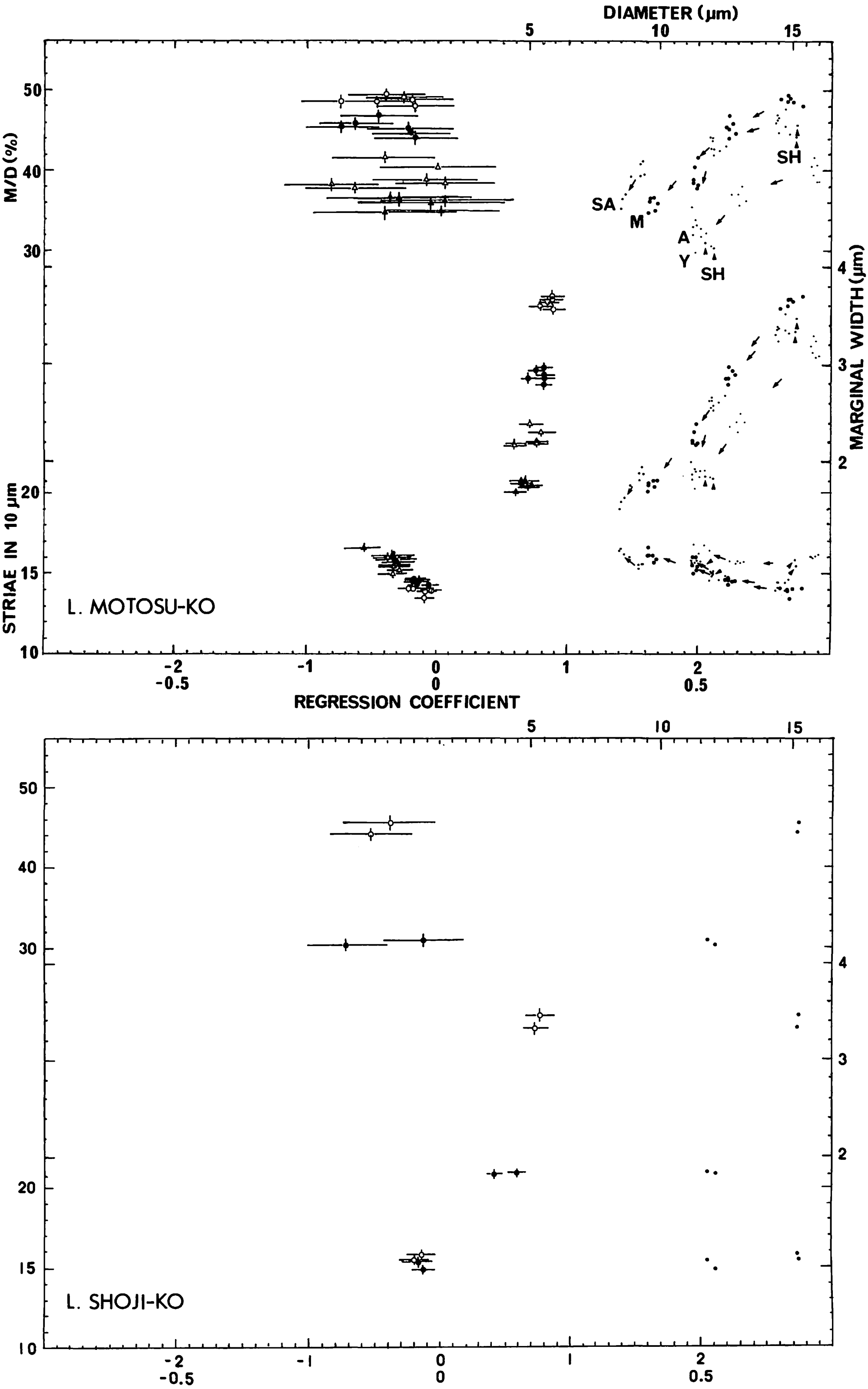

DIAMETER (μm)
5
10
15
50
40
30
20
15
10
M/D (%)
STRIAE IN 10 μm
MARGINAL WIDTH (μm)
4
3
2
SA
M
A
Y
SH
SH
L. MOTOSU-KO
-2
-0.5
-1
0
0
1
2
0.5
REGRESSION COEFFICIENT
5
10
15
50
40
30
20
15
10
4
3
2
L. SHOJI-KO
-2
-0.5
-1
0
0
1
2
0.5

Fig. 4.
A, B

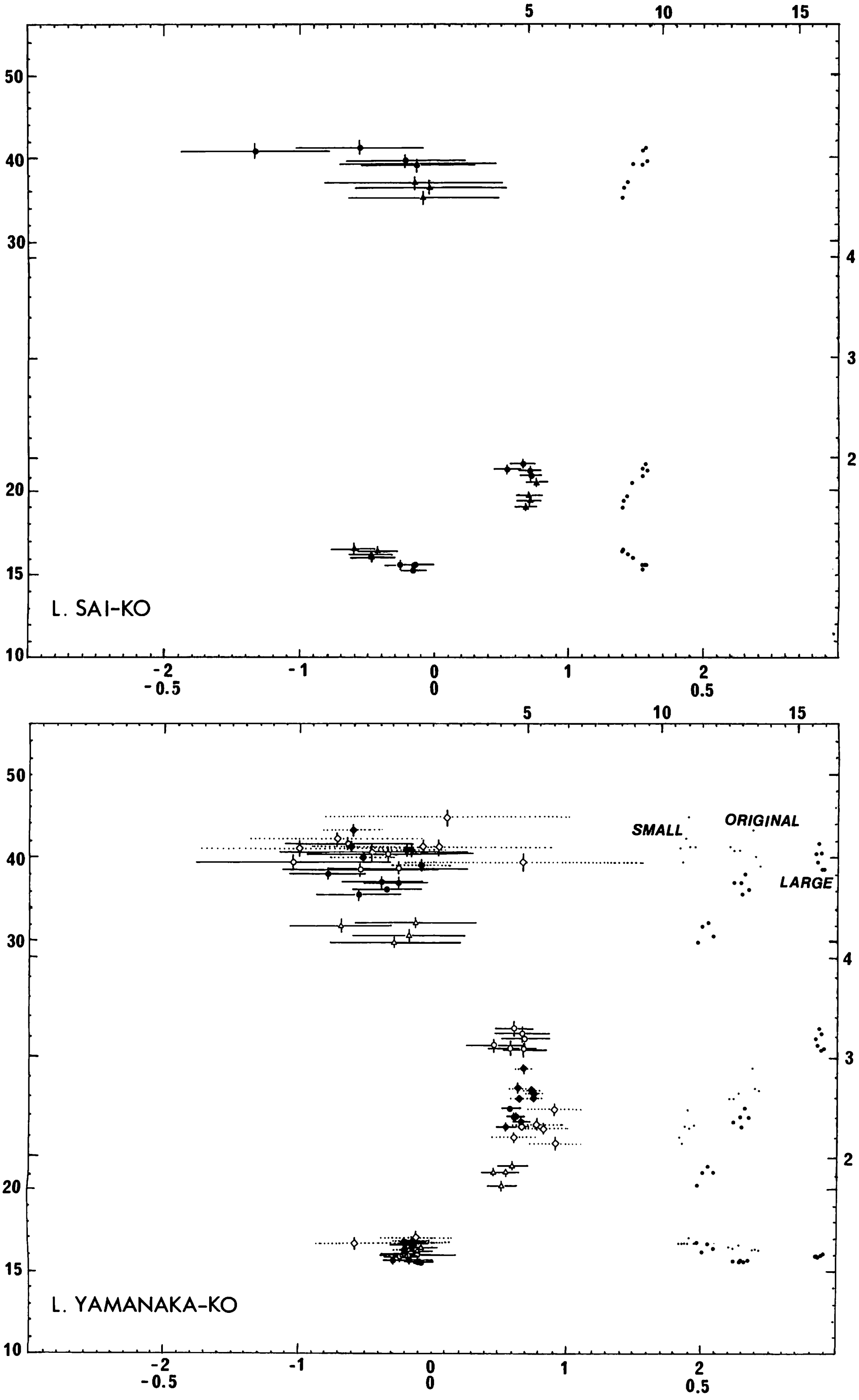

L. SAI-KO
L. YAMANAKA-KO
SMALL
ORIGINAL
LARGE
5 10 15
50 40 30 20 15 10
4 3 2
-2 -1 0 1 2
-0.5 0 0.5

Fig. 4.
C, D

whereas group II is separated from other two groups as it has a low density of alveolar on the valve surface.

Differences in cell density

The cell density decreased in the following order; highest with a spring pulse in Lake Shoji-ko (Group I), with a midsummer pulse in Lake Motosu-ko, with an autumn pulse in Lake Sai-ko, with an early summer pulse in Lake Shoji-ko (Group II), with an obscure pulse in Lake Ashino-ko and lowest with an obscure pulse in Lake Yamanaka-ko (Fig. 3).

Differences in environmental conditions

The point and 95% confidence interval estimates of means of the water temperature (Tw), pH, EC_{25}, T-N, T-P, SiO_2, and COD of the surface water in each season in six lakes are shown in Fig. 6. The interval estimates of the environmental factors within lake in every season were small except for pH and T-P in Lake Shoji-ko in summer. Tw shows the same type of annual change with the highest in summer. The level of pH rises in early summer in Lakes Shoji-ko, Kawaguchi-ko and Ashino-ko, in midsummer in Lakes Sai-ko and Motosu-ko, and in autumn in Lake Yamanaka-ko. EC_{25} is about the same all year round. T-N is high in spring in Lakes Sai-ko, Ashino-ko and Motosu-ko, high in summer and autumn in Lakes Shoji-ko and Kawaguchi-ko, and constant in Lake Yamanaka-ko. No fixed pattern of change

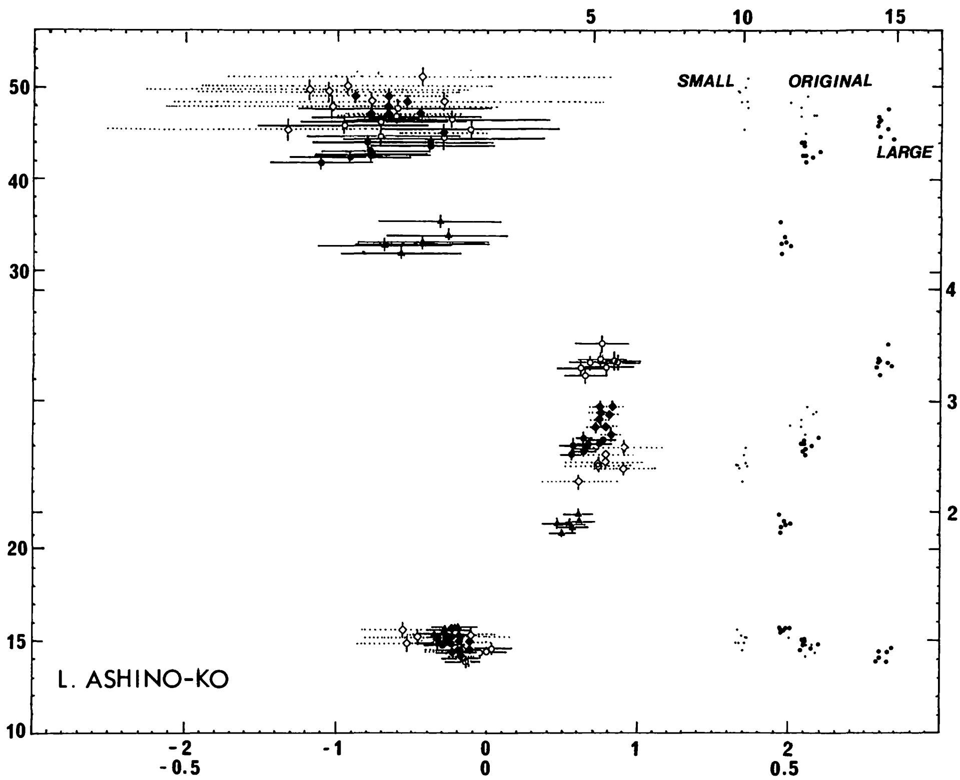


Fig. 4. E

Fig. 4 The point and 95 per cent interval estimates of β and γ of the regression line of M (middle), S (lower) and M/D (upper) on D in April (open circle), June (solid circle), August (open triangle) and October (solid triangle). The relations between D and M, S and M/D expressed by the point estimates of means are shown at the right portion (large dotts for each lake). Original populations are shown by solid diamond and dotted line. Small cell populations are shown by open diamond and dotted line. β ($-2\sim2$) for D–M/D and that ($-0.5\sim0.5$) for D–M and D–S are in the lower abscissa. The upper abscissa is for D in μm. The paths in other lakes are also shown by small dotts in Fig. 4A. M for Lake Motosu-ko, SH for Lake Shoji-ko, SA for Lake Sai-ko, Y for Lake Yamanaka-ko, and A for Lake Ashino-ko.

Table 4. The correlation coefficients of D–M, D–S and D–M/D in the Fuji Five Lakes and Lake Ashino-ko in 1987.

Lakes & Seasons	D–M	D–S	D–M/D
L. Motosu-ko			
Apr.	0.79–0.83	−0.33–−0.07	−0.32–−0.08
June	0.80–0.82	−0.27–−0.09	−0.33–−0.07
Aug.	0.68–0.78	−0.38–−0.31	−0.29– 0.02
Oct.	0.62–0.74	−0.41–−0.24	−0.10– 0.02
L. Shoji-ko			
Apr.	0.71–0.74	−0.23–−0.18	−0.23–−0.15
June	0.63–0.74	−0.21–−0.17	−0.31–−0.05
L. Sai-ko			
June	0.61–0.80	−0.27–−0.14	−0.32–−0.07
Oct.	0.63–0.72	−0.41–−0.31	−0.03–−0.01
L. Yamanaka-ko			
Apr.	0.82–0.89	−0.40–−0.24	−0.37–−0.06
S*	0.58–0.72	−0.38–−0.08	−0.24– 0.16
L*	0.43–0.62	−0.30–−0.08	−0.33–−0.09
June	0.66–0.80	−0.39–−0.12	−0.34–−0.12
Aug.	0.56–0.61	−0.20–−0.08	−0.25–−0.04
L. Ashino-ko			
Apr.	0.83–0.88	−0.41–−0.24	−0.40–−0.14
S*	0.42–0.63	−0.34–−0.08	−0.21–−0.05
L*	0.57–0.71	−0.25– 0.05	−0.30–−0.04
June	0.68–0.73	−0.37–−0.19	−0.40–−0.12
Oct.	0.54–0.68	−0.37–−0.21	−0.21–−0.09

*S, small cell populations; L, large cell populations.

was found out in the other elements.

The difference in Tw between low level (Lakes Motosu-ko, Sai-ko, Yamanaka-ko and Ashino-ko) and high level (Lakes Shoji-ko and Kawaguchi-ko) was remarkable from spring to early summer. It seems to depend on the difference of depth or altitude where the lakes stand. The level of pH was lower in Lakes Motosu-ko and Yamanaka-ko than in the other lakes. The level of EC was low in Lake Motosu-ko, middle in Lakes Shoji-ko, Sai-ko, Yamanaka-ko and Ashino-ko and high in Lake Kawaguchi-ko in all seasons. The high level of EC of water with poor dissolved substances like freshwater lakes implies high concentration of Ca^{2+}, Mg^{2+} or Cl^{-}. These ions are relatively high in Lake Kawaguchi-ko as compared to the other lakes (NAKAJIMA *et al.* 1973). T–N maintained a low level in Lake Motosu-ko and mostly high level in other lakes. The level of T–P was lower in Lakes Motosu-ko and Sai-ko, whereas it was higher in Lakes Ashino-ko, Yamanaka-ko, Kawaguchi-ko and Shoji-ko. The concentration of SiO_2 becomes higher in the order, Lakes Motosu-ko, Shoji-ko, Sai-ko, Kawaguchi-ko, Ashino-ko and Yamanaka-ko. The composition of stratum forming the lake has direct effect upon the concentration of SiO_2 in these lakes. The level of COD was low in Lake Motosu-ko, middle in Lake Sai-ko, and high in Lakes Shoji-ko, Kawaguchi-ko, Yamanaka-ko and Ashino-ko in all seasons except for Lake Sai-ko. Almost all the factors in Lake Motosu-ko are lowest. The level of T–N in Lake Motosu-ko is lower than 0.2 mg/*l* and that of T–P in Lakes Motosu-ko, Sai-ko and Ashino-ko is lower than 0.02 mg/*l*.

Discussion

Two populations in spring in both Lakes Yamanaka-ko and Ashino-ko are sympatric and they differ in the cycle of reproduction. The differences in the range and width of the annual changes of D are significant and the reproduction cycle round might not be an annual periodic phenomenon. Apart from the reproduction cycle, the annual cell cycle can

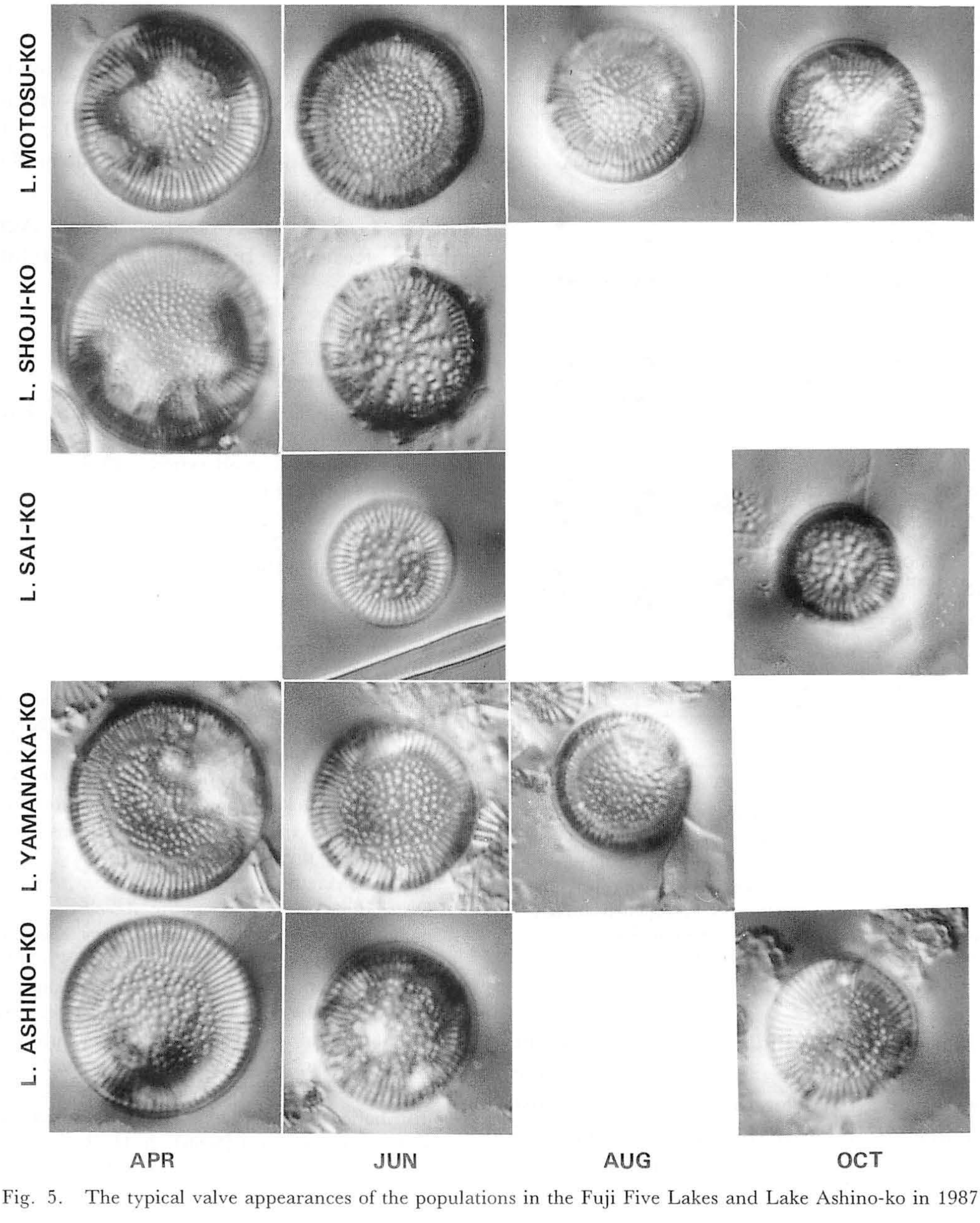


Fig. 5. The typical valve appearances of the populations in the Fuji Five Lakes and Lake Ashino-ko in 1987.

be statistically recognized. This is proved by a fact that M/D in both S and L populations is large.

The form and location of cell size distribution in the population in a batch culture of *Chlorella* changed corresponding to the stage of growth (MARUYAMA, 1977). The cell size distributions in natural population are different from each other in form and location, but whether the difference in distribution is related to the state of multiplication is not clear. The concentrations of T-P and SiO_2 become higher in the order of L. Motosu-ko, L. Ashino-ko, and L. Yamanaka-ko. It is significant whether a definite order of the shift of paths is affected by the concentrations of T-P and/or SiO_2 or not. The cell types have the characters defined by two variables

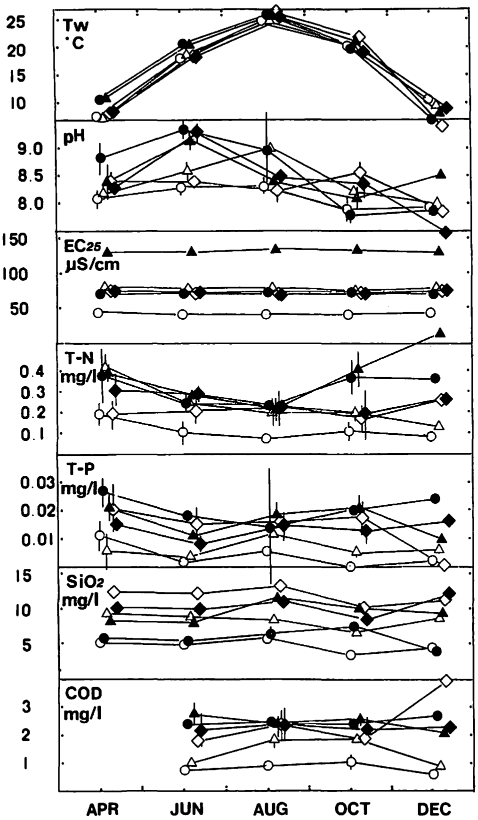


Fig. 6. The point and 95 per cent confidence interval estimates of means of the water temperature (Tw), pH, EC_{25}, T–N, T–P, SiO_2 and COD of the coastal surface water in Lakes Motosu-ko (open circle), Shoji-ko (solid circle), Sai-ko (open triangle), Kawaguchi-ko (solid triangle), Yamanaka-ko (open diamond) and Ashino-ko (solid diamond) in 1987.

such as D-M, D-S and D-M/D, which might be an inherited character. Therefore, some types of taxa of lower rank seem to be most probable. *C. comta* in these lakes is composed of three local taxa of groups I–III, but it is not clear whether diatoms of group III from three localities are identified as the same taxa or not.

The differences in the cell types among lakes and the operation of an annual cell cycle could be identified morphologically in the natural population. Other attributes of cell types and their relation to physico-chemical factors need further understanding.

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猪口眞美*・丸山　晃：富士五湖と芦ノ湖の *Cyclotella comta* 集団の種内差**

1987年，富士五湖と芦ノ湖の沿岸部35地点から5回の採水で得た表層水試料を用いて，*C. comta* 集団の殻面径，縁部幅，条線密度，細胞密度，栄養塩濃度などを測定した。*C. comta* の自然集団は，統計的に，殻面径や珪殻の模様が規則的な年変動をする。同一集団で，殻面径（D）が小さくなれば，縁部幅（M）も狭くなるが，条線密度（S）は大きく，また M/D 比は同じか大きくなる。但し，D–M, D–S, D–M/D の相関係数は，この順に小さくなる。春から秋に向けて，この関係を保ちながら，D, M, と M/D 比は小さい方へ，S は大きい方へ変化する。D, M, S, M/D の湖沼内差は，どの時期でも小さい。D–M, D–S, D–M/D の変数で決められる，湖沼単位で異なる細胞タイプがあるらしい。珪殻のタイプと細胞密度や環境の関わりについても言及した。（*144 東京都大田区西蒲田5–23–22　日本工学院専門学校　**113 東京都文京区弥生1-1-1　東京大学応用微生物研究所）