Autecological Studies on the Marine Tube-Dwelling Diatom *Berkeleya obtusa* (GREV.) GRUNOW*

By

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I Introduction

Numerous papers on the ecology of the marine planktonic diatoms have been published. The influence of various environmental factors on the growth or the survival of the planktonic diatoms have been investigated *in situ* and in laboratory. A large number of abiotic and biotic factors have been proposed to account for the seasonal succession and the occurrence of planktonic diatoms (see GUILLARD and KILHAM 1977). Many floristic studies on the marine littoral diatoms have been carried out all over the world (see MCINTIRE and MOORE 1977) and the seasonal succession and the occurrence of the individual species have been elucidated. In marine littoral diatoms, as well as marine planktonic diatoms, seasonal succession or the occurrence of the individual species should be regulated by various abiotic and biotic environmental factors.

ALEEM (1950) studied the ecology of the marine littoral diatoms at Swanage Bay, England. COX (1977) studied 9 species of tube-dwelling diatoms in the Severn estuary, England over a period of two years and demonstrated the seasonal occurrence of these species. I have confirmed the seasonal occurrence of the marine tube-dwelling diatom *Berkeleys obtusa* (GREV.) GRUNOW at Oshoro Bay, Hokkaido (MIZUNO 1979). However, I could not find the factor(s) regulating its occurrence.

The marine littoral zone is an ecosystem characterized by a changeable habitat because it is the border zone between the marine and terrestrial environment. This zone exhibits the characteristics of terrestrial and marine environments at low water and high water, respectively. Moreover, at low tide, water in tide pools is characterized by extreme changes in temperature, salinity, pH, dissolved O_2 and organic matter (PYEFINCH 1943, ALEEM 1950, CONOVER and SIEBURTH 1966, EDELSTEIN and MCLACHLAN 1975).

IWASAKI (1969) has proposed the following to explain the mechanism of the occurrence of plankton: observations of their behavior, life-history and physiological characteristics

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and measurement of the environmental factors. Furthermore, he has noted that finally it is necessary to evaluate all of these points. IWASAKI's proposal is applicable as method for the study on autecology of marine littoral diatoms.

The present studies were undertaken, initially, with field observations of *Berkeleya obtusa* in the intertidal of Charatsunai, Muroran, Hokkaido. In parallel with these observations, the variation of selected environmental factors in the study area had been measured at regular intervals (MIZUNO 1984a). The influences of the selected environmental factors on the growth or the survival of *B. obtusa* were then examined in culture experiments. Fianlly, the factors controlling the occurrence of this diatom are discussed utilizing the results of the field and culture studies.

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II Seasonal Occurrence and Vertical Distribution of the Marine Tube-Dwelling Diatom *Berkeleya obtusa* in the Intertidal at Charatsunai, Muroran

Several workers have studied marine littoral diatom populations throughout the year and have shown that the marine littoral diatoms exhibit seasonal fluctuations and vertical zonation, as do marine macrolagae (ALEEM 1950, CASTENHOLZ 1963, 1967, HENDEY 1964, HOPKINS 1964, COX 1977, MIZUNO 1977, 1979, 1984b). COX (1977) studied 9 species of the tube-dwelling diatoms for two years and found each species exhibited certain seasonal fluctuations. Environmental changes are thought to be related to these seasonal fluctuations and the vertical distribution of marine littoral diatoms (ALEEM 1950, CASTENHOLZ 1963, 1967, HOPKINS 1964, MCINTIRE and WULFF 1969, WULFF and MCINTIRE 1972).

There are few reports on the ecology of the tube-dwelling diatom *Berkeleya obtusa* (GREV.) GRUNOW (SMITH 1856, MIZUNO 1979). I have documented the seasonal occurrence of *B. obtusa* at Oshoro Bay, Hokkaido, where it occurs from winter to spring, but

abruptly disappears in April (MIZUNO 1979). However, the factor(s) regulating the seasonal occurrence of this diatom remains to be solved. The present study reveals the seasonal occurrence and vertical distribution of *B. obtusa* in the intertidal at Charatsunai, Muroran, Hokkaido and discusses influences of environmental factors on its occurrence.

Materials and Methods

Charatsunai is located in Muroran (42° 19′ N; 141° 59′ E) at the northern part of the mouth of Uchiura Bay (Volcano Bay). The landward part of the central flat shore of Charatsunai was chosen as the study area. From the observation of substrates of *B. obtusa*, this diatom was found on macroalgae with spinous blades, and abundantly on *Sargassum confusum* C. AGARDH, *Sargassum thunbergii* (MERTENS) O. KUNTZE and *Neorhodomela aculeata* (PEREST.) MASUDA, and rarely on *Analipus japonicus* (HARVEY) WYNNE and *Gymnogongrus flabelliformis* HARVEY at this location. Accordingly, the seasonal fluctuations of *B. obtusa* on the first three of these macroalgae were investigated.

Two sampling points were selected in each macroalgal patch (1-5 in Text Fig. 1). These sampling points were located around the Cleft-1 where seawater was sampled for the measurement of environmental factors in my previous study (MIZUNO 1984a). These sam-

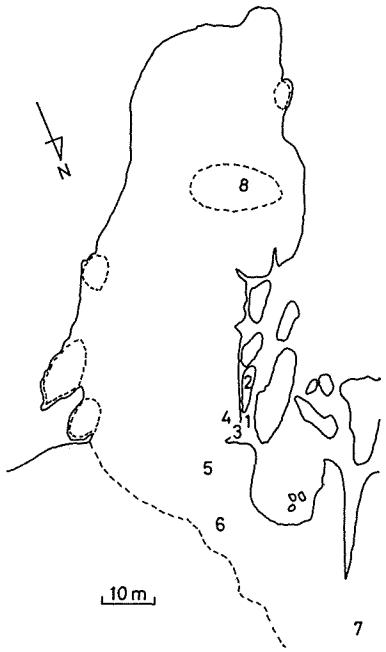


Fig. 1 The sampling points of *Berkeleya obtusa*. 1: *Sargassum confusum*-1 and *Sargassum thunbergii*-1 sampling points; 2: *S. thunbergii*-2; 3: *S. confusum*-2; 4: *Neorhodomela aculeata* -1; 5: *N. aculeate*-2; 6: Pool-2; 7: Pool-3; 8: Pool-4.

pling points ranged between +10 cm and +50 cm of the tidal datum line (the tidal datum line is near the level of the lowest low water of spring tide). Five individuals of *S. thunbergii* and *N. aculeata* or three or one of *S. confusum* were harvested at each sampling point. The Pools 2-4 in my previous study (MIZUNO 1984a) were also chosen as sampling points of *B. obtusa*. The Pool-2 was located at the most landward part of the central flat (6 in Text Fig. 1). It was 0.15 m deep and measured 1.5×1 m and was located at +60 cm above the tidal datum line. The bottom of this shallow pool was sandy mud, but was mostly covered with *N. aculeata*. The Pool-3 was a shallow, small bare rock pool in rugged shore (7 in Text Fig. 1). It was 0.1 m deep, measured 0.6 m×0.4 m, and was located at +80 cm level of the tidal datum line. The Pool-4 was a shallow pool on the central large rock of the central flat (8 in Text Fig. 1), was 0.1 m deep and measured 2 m×1.5 m. This pool was located at +160 cm above the tidal datum line. *S. thunbergii*, *Corallina pilulifera* POSTELS ET RUPRECHT and a sea mussel grew there. *B. obtusa* was harvested within a quadrat (25 cm×25 cm) set in the Pools 2-3 and a small one (10 cm×10 cm) set in the Pool-4. The samplings were carried out monthly from January 1976 to April 1979.

After sampling, the colonies of *B. obtusa* were separated from other tube-dwelling diatoms and filamentous brown algae on the basis of the colonial feature and were put into a bottle containing tap water. Then, a little concentrated HNO_3 and HCl were added to the bottle and the bottle heated to dissolve the gelatinous matrix of the colony. After the bottle cooled, tap water was poured into the bottle and its volume adjusted to 200 m*l*, or to 10 or 50 m*l* when diatoms were not abundant. Diatoms were counted using a hemacytometer and the total diatom cell number in the bottle was calculated. When cell density was too high to count, the sample was diluted with tap water to adequate cell density before counting. To determine the frequency of *B. obtusa*, a part of sample was transferred to a centrifuge tube and was heated with concentrated HNO_3 and HCl. The acid-treated material was washed several times with distilled water and mounted in Pleurax. Under a light microscope, at 1,000x, 200 valves of diatoms were examined and frequency (%) of *B. obtusa* was estimated. Cell number of *B. obtusa* was calculated with the following equation:

$$CN = TC \times F/100$$

where CN was cell number of *B. obtusa*, TC was total cell number of diatom and F was frequency (%) of *B. obtusa*. The standing crop at the sampling points except for Pools 2-4 was calculated with the following equation:

$$SC = CN/L$$

where SC was standing crop of *B. obtusa* and L was total length of 3 or 5 individuals of the host alga. The length of main axis of *S. thunbergii*, the length of the main axis and branches more than 3 cm in length of *N. aculeata*, and the length of the main axis, and branches and blades more than 5 cm in length of *S. confusum* were measured. Standing crops at the Pools 2-4 were calculated from the following equation:

$$SC=CN/A$$

where A was area of a quadrat.

Valve length of 100 valves in each sample collected at four locations of *Neorhodomela aculeata*-1 sampling point, *Sargassum confusum*-1, *S. thunbergii*-1 and Pool-2 from November 1977 to April 1978 was measured under the light microscope with a screw micrometer at 1,000x magnification.

B. obtusa grew together with many macroalgae at the Charatsunai location. It has been found that substances excreted from macroalgae influence the growth of other organisms (YENTSCH and REICHERT 1962, MCLACHLAN and CRAIGIE 1964, CONOVER and SIEBURTH 1966, BERGLUND 1969). The first step of investigating the influence of biological factors on the occurrence of *B. obtusa*, was to measure the standing crops of macroalgae monthly at the central flat at Charatsunai from June 1976 to May 1977. Two 20 m nylon tape measures were laid out at the shore (Text Fig. 2). Ten 25 cm×25 cm (625 cm^2) quadrats were placed on the flat at 5 m intervals along the tape line. All plants found in the ten quadrats were collected and each species weighed in a fresh condition.

From October 1978 to September 1979, the 2 week production rate of *B. obtusa* on *N. aculeata* was examined in the field. The main axis of *N. aculeata* was cut down to a length of 5 cm from the base and all lateral branches removed. This residual axis of the alga was cleaned by removing epiphytes and small animals. Five individuals were prepared in the

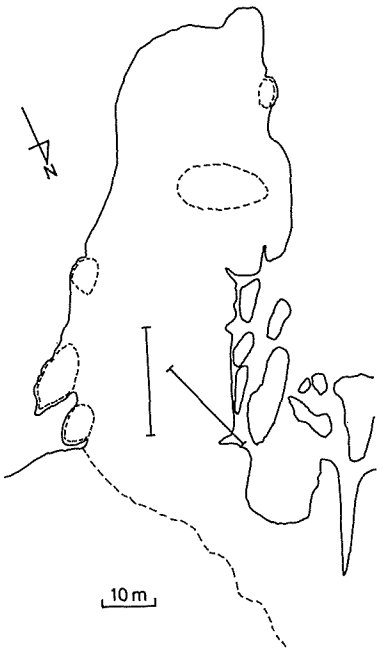
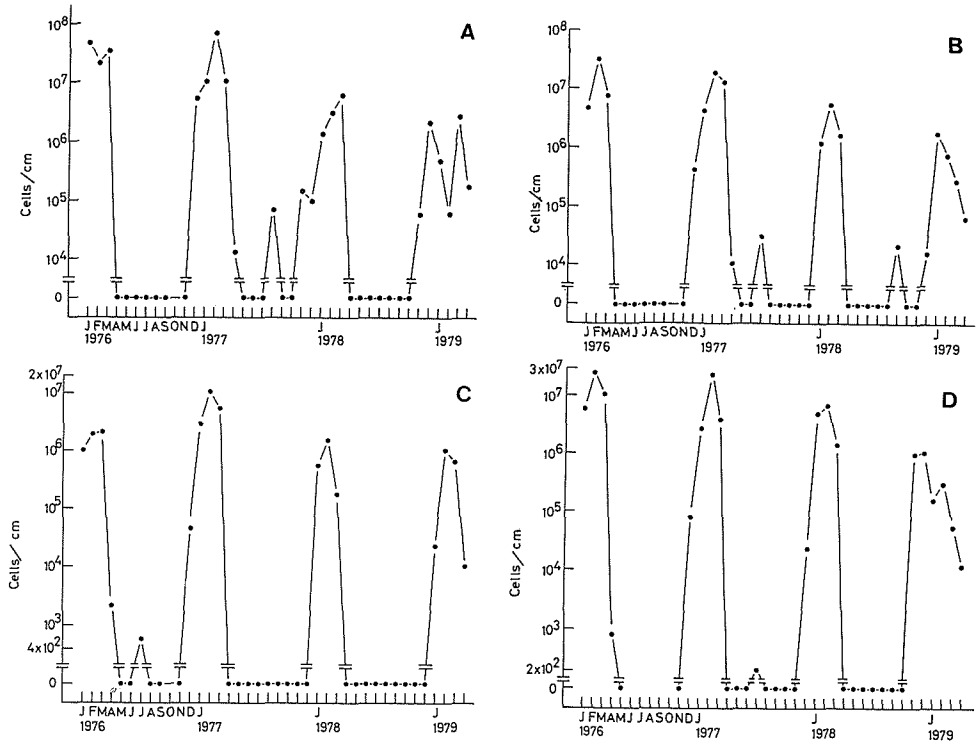


Fig. 2 Locations of two tape lines for sampling of macroalgae.

same way at one station. Three nails with tape were driven into rock around the station as a mark. This experiment was undertaken at two stations at +40 cm above the tidal datum line near the *N. aculeata*-1 sampling point. After 2 weeks, the 5 individuals of *N. aculeata* were harvested and cell number of *B. obtusa* was determined by the same method used for the standing crop measurement.

From December 1979 to January 1980 the timing of the appearance of *B. obtusa* on algal substrates was carried out *in situ*. Two individuals of *S. confusum* and *S. thunbergii* and four individuals of *N. aculeata* growing at the shore were cleaned by removing epiphytes and small animals. Three nails with tape were driven into rock around each observing individual as a mark. The appearance of *B. obtusa* on each individual was observed at intervals of 1-2 days.

The examination of twining of colonies of *B. obtusa* around an artificial substrate was done in laboratory. Five incised polyethlene rods, 3 cm in length, and five smooth-surface rods were prepared (Pl. III, Fig. A). Six hundred m*l* of natural seawater and the two kinds of rods were placed in a one liter beaker and stirred for 5 minutes together with a large number of colonies of *B. obtusa* collected from the field.



Results

Seasonal fluctuation of B. obtusa. The colonies of *B. obtusa* attached to the surface of the natural substrates (Pl. I). *B. obtusa* did not occur in the Pools 3-4 during the experimental period. At the other seven sampling points, clear seasonal occurrence was observed (Text Fig. 3). At these sampling points, *B. obtusa* began to occur in November or December and increased to the annual maximum of $1\times10^6-7\times10^7$ cells/cm or $2\times10^5-5\times10^6$ cells/cm^2 (for the Pool-2) during January and March and abruptly decreased from March or April. *B. obtusa* was not observed at any sampling locations from May to October except for sporadic occurrences in the period from July to September. These sporadic occurrences did not occur at the *S. thungergii* sampling locations or in Pool-2. The standing crop during these sporadic occurrences ranged from 6×10^2 to 7×10^4 cells/cm and was less than that of the main occurrences from November to April. The duration of these sporadic occurrences was short. The results of the measurements of macroalgal standing crops at the central flat show the same seasonal pattern as *B. obtusa* at the above mentioned seven sampling points. *B. obtusa* began to occur in October, was abundant from December to March and disappeared from April to September (Table 1).

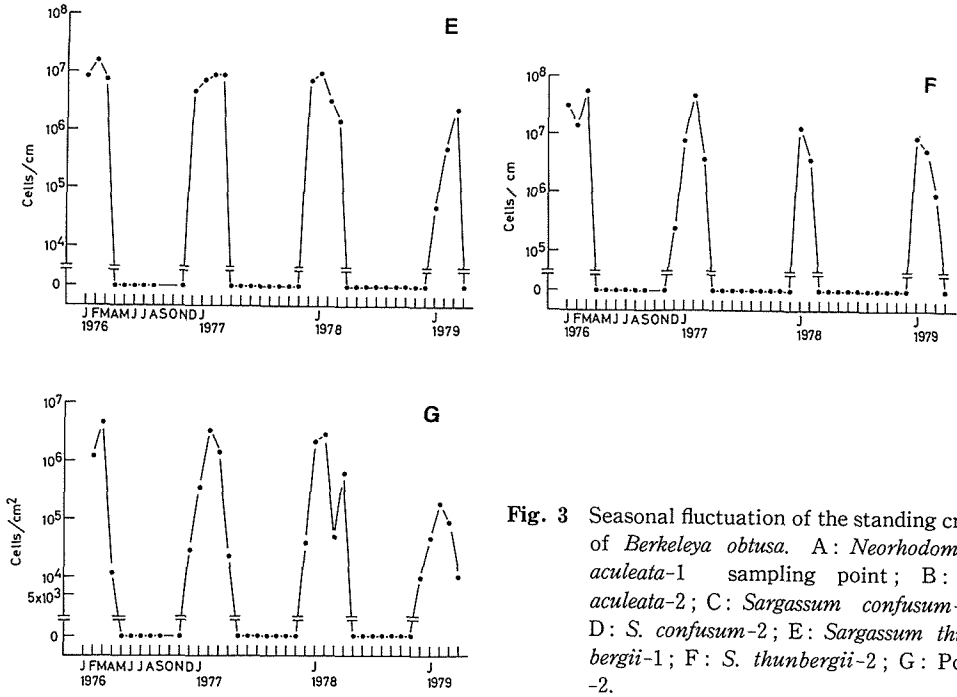


Fig. 3 Seasonal fluctuation of the standing crop of *Berkeleya obtusa*. A: *Neorhodomela aculeata*-1 sampling point; B: *N. aculeata*-2; C: *Sargassum confusum*-1; D: *S. confusum*-2; E: *Sargassum thunbergii*-1; F: *S. thunbergii*-2; G: Pool-2.

Table 1 Seasonal fluctuations in standing crop (fresh weight g/625×10 cm²) of *Berkeleya obtusa* and macro-plants.

Month	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Species												
Bacillariophyceae												
Berkeleya obtusa	0	0	0	0	1	4	67	48	99	17	0	0
Chlorophyceae												
Kornmannia zostericola	0	0	0	0	0	0	0	58	29	16	38	9
Monostroma angicava	0	0	0	0	0	0	0	0	15	17	23	43
Ulva pertusa	215	126	221	162	217	103	45	40	40	16	58	40
Phaeophyceae												
Alaria crassifolia	23	50	45	0	105	9	72	0	0	23	51	64
Analipus japonicus	29	3	0	0	0	0	0	0	3	19	46	39
Colpomenia bullosa	0	0	0	0	0	0	3	5	61	62	27	3
Dictyopteris divaricata	6	24	1	0	0	0	0	0	0	1	0	0
Dictyota dichotoma	0	28	80	28	35	11	0	0	0	0	0	0
Laminaria japonica	347	187	650	118	37	78	103	1	0	9	216	244
Leathesia difformis	103	66	1	0	0	0	0	0	0	0	0	5
Sargassum confusum	52	116	106	156	132	196	303	128	128	131	71	110
S. thunbergii	18	25	25	18	2	4	0	0	0	0	0	6
Scytosiphon lomentaria	37	0	0	0	0	0	0	0	3	6	27	11
Rhodophyceae												
Neodilsea yendoana	2	0	0	0	2	1	10	1	4	50	20	22
Neorhodomela aculeata +epiphytes[1)]	306	236	193	72	262	543	555	348	356	382	233	334
Palmaria palmata	0	0	0	0	0	0	0	24	67	226	111	31
Angiosperm												
Phyllospadix iwatensis	125	102	163	119	90	230	58	151	142	50	77	127
Others	50	38	24	18	22	23	21	6	3	22	3	18
Mean (g/625 cm²)	131	100	151	69	90	120	124	81	95	105	100	111

[1)] Epiphytes were *Sphacelaria* sp., *Ceramium* sp., *Polysiphonia morrowii*, *Symphyocladia latiuscula*, etc.

Auxospore formation and change in the size distribution of B. obtusa *at four sampling points.* Auxospore formation was observed once in the field sample of October 1978. The auxospore formation took place within the colonial tube. Two auxospores were produced from two mother cells (gametangia). The valve length of gametangia ranged from 18.3 to 20.9 μm and its mean±SD was 19.8±0.8 μm (n=30). The valve length of the auxospore initial cells ranged from 31.6 to 38.5 μm and its mean±SD was 35.6±1.9 μm (n=30).

Text Figure 4 shows the monthly change in the size distribution from November 1977 to April 1978 at four sampling locations. A bimodal frequency distribution was often ob-

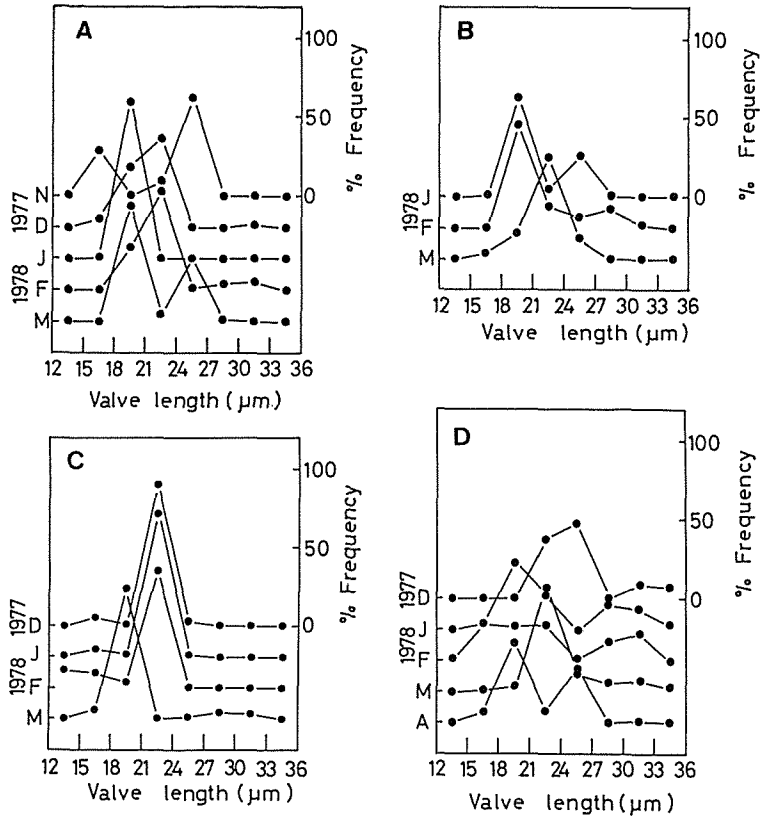


Fig. 4 Monthly change in frequency distribution of length of *Berkeleya obtusa* collected from four sampling locations from November 1977 to April 1978. A: *Neorhodomela aculeata*-1 sampling point; B: *Sargassum confusum*-1; C: *Sargassum thunbergii*-1; D: Pool-2.

served. The pattern of frequency distribution varied with the sampling location. Modal values at the initial stage of occurrence were a little larger than those at the final stage of occurrence at three sampling points, while the modal value for *S. confusum*-1 at the initial stage was a little smaller than that at the final stage. Many cells of 18-21 μm in length, which was within the size range of reproductive cells, were observed in many samples. A few large cells of more than 30 μm in length, which was near the lower limit of the initial cells size, were observed.

Seasonal fluctuation in the length of the host algae. Text Figure 5 shows the seasonal fluctuations of the mean length of host algae at *N. aculeata*-1, *S. confusum*-1 and *S. thunbergii*-1 sampling locations. All host algae were perennial. The minimum of length of the algae occurred in winter and they scarcely grew during this season. In spring they began to grow and reached the annual maximum length in summer. Upright thalli of *S. confusum*

and *S. thunbergii* fell off during summer-autumn and the algal lengths in autumn became similar to the winter values. The upright thalli of *N. aculeata* fell off from summer to early winter.

Seasonal fluctuation in standing crop of macro-plants at the central flat. The species whose standing crop was more than 20 g per ten quadrats was listed in Table 1. *Ulva pertusa* KJELLMAN, *Sargassum confusum*, *Neorhodomela aculeata* and *Phyllospadix iwatensis* MAKINO occurred throughout the year. *Alaria crassifolia* KJELLMAN and *Laminaria japonica* ARESCHOUG occurred throughout the year except winter. *Kornmannia zostericola* (TILD.) BLIDING, *Colpomenia bullosa* (SAUND.) YAMADA and *Palmaria palmata* (L.) O.

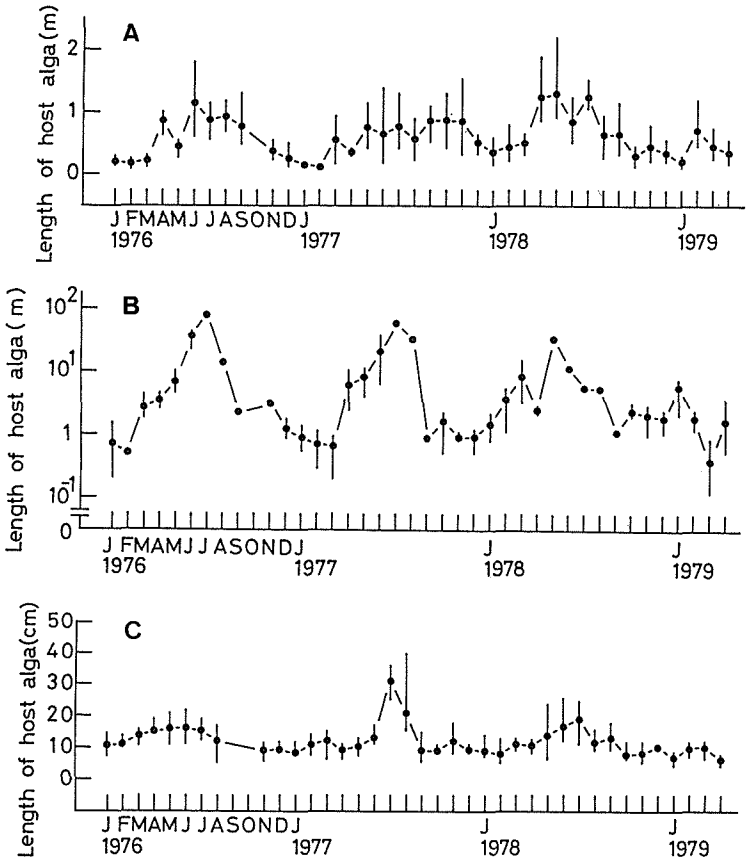


Fig. 5 Seasonal fluctuation in the mean length of the host alga of *Berkeleya obtusa*. A: *Neorhodomela aculeata* at *N. aculeatea*-1 sampling station (n=5 individuals); B: *Sargassum confusum* at *S. confusum*-1 sampling station (n=1 or 3 individuals); C: *Sargassum thunbergii* at *S. thunbergii*-1 sampling station (n=5 individuals). Vertical bar shows the range between the maximum and minimum length. For detailed method of measurement of length see in the text of "Materials and Methods" of the Chapter II.

KUNTZE grew abundantly from winter to spring. *Monostroma angicava* KJELLMAN, *Analipus japonicus*, *Scytosiphon lomentaria* (LYNGB.) LINK and *Neodilsea yendoana* TOKIDA grew abundantly in spring or from spring to early summer. *Dictyopteris divaricata* (OKAMU.) OKAMURA, *Dictyota dichotoma* (HUND.) LAMOUROUX, *Leathesia difformis* (L.) ARESCHOUG and *Sargassum thunbergii* grew abundantly in summer or from summer to autumn.

Production rate of B. obtusa *on* N. aculeata. The production rate of *B. obtusa* on *N. aculeata* for 2 week intervals in the field is shown in Table 2. *B. obtusa* was found on *N. aculeata* from late October 1978 to early March 1979, but it was not found from mid March 1979 through September 1979. From October to December 1978, *B. obtusa* was observed at one or the other station. From January through February 1979, *B. obtusa* was observed at both stations, while in early March 1979 it was observed only at station-2. High production rates of $1\text{-}8\times10^5$ cells/cm were observed at biweekly intervals in November and from January to early March. *B. obtusa* was observed at the station-1 in the October 1978 sample, but was not observed in the *N. aculeata* samples collected at the same sime (Text Fig. 3A, B). *B. obtusa* occurred in several samples in April 1979, but it did not attach to the substrate at either stations after mid March 1979.

Attachment of B. obtusa *to substrates*. Observations on the appearance of *B. obtusa* on macroalgae showed that various sized colonies appeared on all individuals of *S. confusum* examined, one of two individuals of *S. thunbergii* and one of four individuals of *N. aculeata* within 1-2 days (Pl. II). A large colony, easily visible with naked eye, appeared for only 1-2 days. These tubular colonies always coiled around the substrates or grew between the spines of the substrates.

Laboratory experiment with colonies of *B. obtusa* showed that colonies could coil around and attach to an incised rod (Pl. III, C) but not to smooth-surface rod (Pl. III, B). The colonies were retained by the gaps in the incised rod (Pl. III, D-F).

Monthly changes in epiphytic diatoms on the surface of colonial tube of B. obtusa. Epiphytic diatoms were rarely observed on the surface of the colonial tube of *B. obtusa* in January (Pl. IV, A, D, G). However, in February and March, epiphytic diatoms belonging to the genus *Fragilaria*, *Licmophora*, *Melosira*, *Navicula*, *Synedra*, etc. grew abundantly on the surface of the colonial tubes (Pl. IV, B-C, E-F, H-I).

Discussion

SMITH (1856) reported that *Schizonema obtusum* GREV. (=*B. obtusa*) grew on large algae. In my previous study (MIZUNO 1979), *B. obtusa* was found on macroalgae and sea mussels in Oshoro Bay. At Charatsunai, *B. obtusa* selectively attaches to certain macroalgae and does not grow on any sea mussels, rocks and concrete blocks. Laboratory experiments with twining colonies show that colonies can only twine around (*i. e.* attach) to a substrate bearing spine-like structures. Observation of *B. obtusa* on macro-algal substrates

in short term *in situ* experiments shows that *B. obtusa* appears on these substrates in a colonial mass. It is difficult to believe that solitary cells grow to the large visible colonies in only one or two days, judging from the culture experiment of the present diatom. Colonies of this diatom can initially coil around spinous substrates and then be caught hold of in gaps of the substrates, later attaching to the substrates. At Charatsunai, the growing zone of sea mussels is exposed to strong waves. Colonies of the present diatom might be washed away by strong waves before it can attach to sea mussels.

Benthic marine diatoms exhibit the seasonal fluctuation and vertical distribution as do macroalgae (ALEEM 1950, CASTENHOLZ 1963, 1967, HENDEY 1964, HOPKINS 1964, MCINTIRE and WULFF 1969, WULFF and MCINTIRE 1972, COX 1977, MIZUNO 1977, 1979, 1984b). *B. obtusa* shows the seasonal fluctuation and vertical distribution at Charatsunai as well as at Oshoro (MIZUNO 1979). The annual maximum of the standing crop occasionally occurred in March. However, the production experiment (Table 2) shows that this diatom did not appear on the substrate after mid March. Thus shore environment seems to become unfavorable for the occurrence of the diatom after mid March. The standing crop and production rate data show that the environment from November to early March is favorable for its occurrence. At Charatsunai, *B. obtusa* is a dweller of the lower littoral zone (below +60 cm of the tidal datum line).

Table 2 Production rate per 2 weeks of *Berkeleya obtusa* on *Neorhodomela aculeata* in the field. Values are cell number per 1 cm of *N. aculeata*.

Experimental period	Cell number per 1 cm of *N. aculeata*	
	Station-1	Station-2
17 Oct.1978 — 31 Oct.	32,666	0
1 Nov. — 15 Nov.	339,163	0
15 Nov. — 29 Nov.	8,571	80,965
30 Nov. — 14 Dec.	0	3,683
14 Dec. — 28 Dec.	0	35,200
28 Dec. — 11 Jan. 1979	129,776	240,798
12 Jan. — 26 Jan.	252,131	340,469
26 Jan. — 9 Feb.	118,488	861,214
9 Feb. — 23 Feb.	269,386	9,222
23 Feb. — 9 Mar.	0	257,553
16 Mar. — 30 Mar.	0	0
16 Apr. — 30 Apr.	0	0
30 May. — 13 Jun.	0	0
8 Aug. — 22 Aug.	0	—[1]
7 Sep. — 21 Sep.	0	0

[1] No sampling because of the loss of the mark.

Berkeley rutilans (TRENT.) GRUNOW, morphologically allied to *B. obtusa*, was observed throughout the year at Charatsunai and mainly in the zone of upper littoral (above + 80 cm of the tidal datum line-(MIZUNO 1984b)). *B. obtusa* also exhibits a different seasonal cycle and vertical distribution than *B. rutilans*.

ALEEM (1950) believed that prolonged intense insolation during emersion severely desiccated the algae and caused their disappearance during summer. CASTENHOLZ (1963) reported that an upper limit of diatom cover and species composition depended principally on the time exposed to direct sunlight during emersion. Moreover, HOPKINS (1964) suggested desiccation by solar radiation and drying wind as the most important factor affecting the seasonal fluctuation of littoral diatoms. HOPKINS also believed that air temperature during emersion and nutrients in seawater affected the seasonal fluctuation of littoral diatoms. CASTENHOLZ (1967) showed that two dominant diatoms in winter greatly decreased on submerged glass plates in March. He explained that this decrease was due to the supra-optimal light intensity of March. MCINTIRE and WULFF (1969) and WULFF and MCINTIRE (1972) also reported that light intensity influenced the vertical distributions of littoral diatoms.

The lower low water of the spring tide occurs in the nighttime from late September to early March and in the daytime from late March to early September at Muroran (MIZUNO 1984a). The period of occurrence of *B. obtusa* coincides approximately with the period when the lower low water of the spring tide occurs during nighttime.

Taking the time of emersion into consideration, the monthly mean air temperature ranges from 1℃ to 5℃ (at 20 : 00 hrs) during the period of initial occurrence (November-December), from −6℃ to −2℃ (at 20 : 00 hrs) during the period of abundance (January-February) and from 7℃ to 13℃ (at 13 : 00 hrs) during the period of decrease (March-April) (MIZUNO 1984a). During the period when *B. obtusa* is not observed (May-October), mean air temperatures range from 11℃ to 29℃ (at 13 : 00 or 20 : 00 hrs). The monthly mean seawater temperatures at 13 : 00 hrs is 8-12℃ during the period of increase, 2-5℃ during the period of abundance, 3-7℃ during the period of decrease, and 9-21℃ when the diatom is absent (MIZUNO 1984a).

At Charatsunai, this diatom occurs below about 10℃. The monthly mean seawater temperatures during the period of decrease is lower than during the period of increase but in similar to that during the period of abundance. However, the seawater temperature of pools in the littoral zone often rose up to 18℃ or more during emersion in the daytime of March-April (MIZUNO 1984a). The field data suggest that this diatom taxon cannot tolerate high air and seawater temperatures.

Taking the time of emersion into consideration again, the monthly mean evaporation is 0.1-0.2 g/28 cm²/hr (in the nighttime) during the periods of increase and abundance, 0.38-0.54 g/28 cm²/hr (in the daytime) during the period of decrease and 0.31-0.88 g/28 cm²/hr (in the daytime) during the period when the diatom is not observed (MIZUNO 1984a).

When present, the diatom in almost certainly exposed to seawater with a pH of about 8 (MIZUNO 1984a). In late March the lower low water occurs in the daytime (MIZUNO 1984a) and the pH rises to about 9 (MIIZUNO 1984a). This rise in pH might be due to photosynthesis of macroalgae (ATKINS 1919-22). This high pH value is observed in the period when the lower low water occurs in the daytime and this period approximately coincides with the period of disappearance of the diatom. These two environmental parameters may also influence the occurrence of the present diatom.

Nutrients concentrations (nitrate, phosphate and silicate) are high in seawater at Charatsunai from late autumn to early spring and low for the remainder of the year (MIZUNO 1984a). The period of occurrence of *B. obtusa* coincides with the period of high concentrations of nutrients in seawater. It is likely that the concentrations of these nutrients affect the occurrence of the diatom.

At Muroran, solar radiation increases from February onward and by March the amount is three times as great as in December or January (UCHIDA 1981). This increment of solar radiation may be related to the decrease of the diatom in March-April.

It is known that brown and red macoalgae produce toxic extracellular substances inhibiting the growth of bacteria and unicellular algae, including a diatom (MCLACHLAN and CRAIGIE 1964, FENICAL 1974, 1975). On the other hand, some green alga liberates organic compounds which stimulate the growth of itself and other species (BERGLUND 1969). Excretion rates of dissolved organic matter by marine seaweeds during the period of good growth were 2.8-3 times as great as during the period of poor growth (KHAILOV and BURLAKOVA 1969). Field observations show the inverse relation between the occurrence of *B. obtusa* and the length of host algae. *Kornmannia*, *Colpomenia* and *Palmaria* begin to grow abundantly during the period of the occurrence of the present diatom. While *Monostroma*, *Analipus*, *Scytosiphon* and *Neodilsea* begin to grow abundantly during the period of its decrease. *Alaria* and *Laminaria* become abundantly during the periods of decrease and disappearance of the diatom and become less abundant during the period of abundance. *Dictyopteris*, *Dictyota*, *Leathesia* and *S. thunbergii* occur abundantly when *B. obtusa* is not present. *Ulva*, *S. confusum*, *Neorhodomela* and *Phyllospadix* occur throughout the year. Fluctuations of macroalgae and changes in excretion rates of organic matter might influence the occurrence of the diatom.

In January, few epiphytic diatoms attach to the surface of the colonial tube of *B. obtusa*, but many diatom species attach and become abundant on the surface in February and March. February and March is the period of the maximum or the decrease in the standing crop of *B. obtusa*. Reduction of nutrient transport and light penetration due to the epiphytic diatom's fouling on the surface of colonial tube might limit the growth of *B. obtusa*.

The observations of auxospore formation and monthly change in size distribution show that the present diatom probably produces auxospores in autumn and winter. Small cells near the lower limit of the size range are less viable (GEITLER 1932, VON STOSCH and

DREBES 1964, PAASCHE 1973). The lower limit of the size of the present diatom is thought to be near 18 μm, since the lower limit of the length of the auxospore mother cell is *ca.* 18 μm. Small cells less than 18 μm long were rarely observed during the decreasing stage of occurrence. A low viability of small cells seems to be not related to the decrease of the diatom in March-April.

The occurrence of *B. obtusa* at Charatsunai is probably controlled by the physical, chemical and biological factors mentioned above.

Many environmental factors influence algal vertical distribution (cf. BIEBL 1962, ZANEVELD 1969). *B. obtusa* is thought to be sensitive to desiccation because its occurrence is restricted to the periods of low evaporation rates when it is exposed to air. When the present diatom grows at a high level of the littoral zone, it might be exposed to atmosphere for a longer time. Even though the evaporation rate is low in the nighttime, *B. obtusa* occurring at a high level in the littoral might be severely desiccated during night-time emersion. Moreover, the zone of the upper +80 cm of the tidal datum line emerges in the daytime during the occurrence period of the diatom at lower levels (JAPAN METEOROLOGICAL AGENCY 1977). Since the evaporation rate of water in the daytime is higher than at nighttime (MIZUNO 1984a), the desiccation during emersion may be one cause of why the present diatom does not grow in the upper zone above +80 cm at Charatsunai.

There is no affect of desiccation in tide pools. However, the environmental parameters such as water temperature, pH and salinity take extreme values in the Pool-4 which is located at the level of +160 cm (MIZUNO 1984a, data for the Pool-4). This severe environment does not allow growth of the diatom. The diatom is not observed in the Pool-3 which is located at the level of +80 cm, even though temperature, pH and salinity during the period of occurrence of the diatom are approximately the same as in the Pool-2 where the diatom occurs (MIZUNO 1984a). CASTENHOLZ (1967) suggests that supra-optimal irradiance causes the decrease of winter diatoms in March. As the present diatom can be regarded as a winter diatom, it might be sensitive to high irradiance. Exposure to supra-optimal irradiance during emersion may prevent its growth. The lack of an suitable substrate is possibly another one of the causes why the diatom does not occur in the Pool-3, since the macroalgae to which *B. obtusa* selectively attaches do not grow there.

ALEEM (1950) suggests that persistence in the sublittoral zone is one of the modes of survival of littoral diatoms during the period of their absence from the shore. In Uchiura Bay (Volcano Bay), a low seawater temperature of nearly 10℃ was recorded in the zone of below 15 m in depth during warm months (OHTANI *et al.* 1971a, 1971b, NISHIHAMA *et al.* 1979). Although a systematic survey of the sublittoral zone was not carried out in the present study, a large number of colonies of the present diatom was observed once on a submerged rope at a depth of 5-10 m at 100 m off Charatsunai in July 1976. Thus *B. obtusa* might grow during warm months in the sublittoral zone where seawater temperature is low.

B. obtusa sporadically occurred during warm months when the shore environment seems

to be unfavorable for occurrence. Observation on twining of colonies *in situ* and in the laboratory has shown that the diatom initially appears on spinous substrates in a large colony. The colonies observed at the shore during warm months are regarded as drifting fragments of colonies which have grown up in the sublittoral zone and which have been transported.

III Culture Studies of *Berkeleya obtusa*

1. Influence of temperature, daylength and light intensity on the growth of *Berkeleya obtusa*, and viability at three different irradiance levels at 14°C

Temperature seems to be one of the important factors governing the seasonal succession of phytoplanktons (see GUILLARD and KILHAM 1977). IGNATIADES and SMAYDA (1970a) demonstrated that *Rhizosolenia fragilissima* BERGON which was the characteristic species of late summer-autumn community in Narragansett Bay grew well at 12-25℃ in culture, and *in situ* and *in vitro* responses of this species to optimum temperature levels were in general agreement. However, DURBIN (1974) showed that *Thalassiosira nordenskiöldii* CLEVE which was one of the major diatoms in winter-spring blooms in Narragansett Bay began rapid growth at 2.45℃ *in situ* while it grew at a maximum rate at 10℃ and relatively high rate at 15℃ in culture. SMAYDA (1969) stated discrepancies between the responses of the field and laboratory studies to optimal temperature levels occurred often.

Daylength and light intensity seem to be important factors governing the seasonal succession of phytoplanktons (see GUILLARD and KILHAM 1977). By the laboratory study, CASTENHOLZ (1964) showed that daylength and light intensity are principal factors controlling the seasonal distribution of marine littoral diatoms.

In the present study, the influences of temperature, day length and light intensity on the growth of *Berkeleya obtusa* have been examined in culture.

In February and March many diatom species attached abundantly to the surface of the colonial tube of *B. obtusa* (Chapter II). The growth rates of four epiphytic diatoms have been examined at three different temperatures and have been compared with the growth rate of *B. obtusa*.

It is known that diatom cells are capable of survival for weeks and even months when they are kept either in dim light or in complete darkness (ANTIA and CHENG 1970. IGNATIADES and SMAYDA 1970b, SMAYDA and MITCHELL-INNES 1974, HELLEBUST and LEWIN 1977). For a photoautotrophic diatom without capability of producing resting spores, it is an ecological problem as to how long a diatom can retain its variability in dim light or the darkness following its removal from the euphotic zone (SMAYDA and MITCHELL-INNES 1974).

B. obtusa disappears from the littoral zone from May to October except for an isolated sporadic occurrence (Chapter II). It is believed that the present diatom probably sinks to

the lower layer of the euphotic zone or below during this period. Thus experiments of its viability were carried out under different illuminating conditions.

Materials and Methods

Growth experiment. *Berkeleya obtusa* collected from Charatsunai in October 1977 was isolated into axenic clonal culture by repeated micropipette washing. Stock and experimental cultures were grown in screw-cap test tubes (1.8 cm×13.5 cm) containing 10 m*l* of medium in temperature controlable incubators. Stock cultures were maintained in a modified artificial seawater medium BSW-2A (UCHIDA 1974) (Table 3) at 14℃ under a 14 : 10 hr LD cycle. Illumination (3,000 lx) was provided by cool white fluorescent lamps. The cultures used in the experiments were maintained in modified BSW-2A where nitrate, phosphate and silicate were added to final concentrations of 0.12 ppm of N, 0.04 ppm of P and 0.5 ppm of Si. These concentrations approximated to the maximum levels found at Charatsunai (MIZUNO 1984a). Other elements were added to the same level as in the modified BSW-2A. Since *B. obtusa* excretes gelatinous materials and forms a colonial mass in a natural seawater medium, but not in an artificial seawater medium, the modified BSW-2A

Table 3 Composition of modified BSW−2 and −2A culture media.

	Modified BSW−2	Modified BSW−2A
	(Amount/100 m*l*)	
NaCl	———	2.4 g
KCl	———	60 mg
Ca as Cl	———	30 mg
$MgSO_4 \cdot 7H_2O$	———	0.6 g
$MgCl_2 \cdot 6H_2O$	———	0.3 g
$NaHCO_3$	———	10 mg
Seawater	80 m*l*	———
$NaNO_3$	5 mg	5 mg
K_2HPO_4	0.5 mg	0.5 mg
$Na_2NiO_3 \cdot 9H_2O$	1−10 mg	1−10 mg
P II metals[1)]	1 m*l*	1 m*l*
S II metals[1)]	1 m*l*	1 m*l*
Thiamine-HCl	10 μg	10 μg
Biotin	0.1 μg	0.1 μg
Vitamin B_{12}	0.2 μg	0.2 μg
Tris (hydroxymethyl) aminomethane	0.1 g	0.1 g
Nitrilotriacetic acid	10 mg	10 mg
pH	7.8—8.0	7.8—8.0

[1)] Provasoli *et al* (1957).

was employed as a medium in this experiment in order to ward off variation of cell density in inoculum and make counting easier.

First, growth was examined under different combinations of temperature and daylength. The temperatures were 5, 10, 14, 18 and 22℃ and with light-dark cycles of 14 : 10 hrs and 10 : 14 hrs. Light intensity was 3,000 lx. Second, growth was examined at various light intensities at 5, 10 and 14℃. The light-dark cycle was 14 : 10 hrs.

The cultures were precultured under each experimental condition for one week. An inoculum of 100-500 cells/m*l* was used. The cells were counted at the intervals of 5-17 days during exponential growth stage using hemacytometer. The growth rate was calculated from the following equation:

$$\mu = \frac{1}{t} \log_2 \left(\frac{Nt}{No} \right)$$

where *Nt* and *No* are the cell concentrations at times t and zero and μ is the growth rate (divisions/day) (EPPLEY 1977).

Four epiphytic diatoms (*Fragilaria capucina* DESMAZ. var. *capucina*, *Melosira* sp., *Navicula* sp. and *Synedra investiens* W. SMITH) growing on the colonial tube of *B. obtusa* were isolated into unialgal clonal cultures from February to March, 1979. Their growth rates were examined by method used for *B. obtusa*. The cells were counted at the intervals of 2-6 days during exponential growth stage using hemacytometer or with a ruled counting plate. Inocula of 10-200 cells/m*l* were used.

Viability experiment. An axenic clone of *B. obtusa* collected from Charatsunai in November 1978 was used in this experiment. The exponentially growing stock culture was washed once with unenriched natural seawater and inoculated into test tubes containing 10 m*l* of unenriched natural seawater. An inoculum of 100 cells/m*l* was used. The test tubes

Table 4 Influence of temperature and daylength on the growth of *Berkeleya obtusa*.

Temperature (℃)	Daylength	Growth rate (divisions/day) ($\bar{X} \pm SD$; n=3)
5	14 : 10 hr LD cycle (L)	0.29±0.06
	10 : 14 hr LD cycle (S)	0.28±0.06
10	L	0.46±0.05
	S	0.45±0.07
14	L	0.59±0.07 (n=2)
	S	0.53±0.03 (n=2)
18	L	0.10±0.07
	S	no growth
22	L	no growth
	S	no growth

were then placed at three irradiance levels at 14℃ with 14 : 10 hr LD cycle. The three irradiance levels were 3,000 lx, 170 lx and complete darkness. The tubes exposed to 170 lx were prepared by covering the test tubes with parchment paper. Darkness was obtained by covering the tubes with aluminum foil. After exposure to these three conditions for 1, 2, 4 and 6 months, about one m*l* of algal suspension was transferred into a test tube containing 10 m*l* of a fresh modified BSW-2 (Table 3) and was incubated at 14℃ during 14 : 10 hr LD cycle at 3,000 lx. The cell concentration in one test tube exposed to each irradiance level was counted using hemacytometer. Viability was judged by the occurrence of brown colonies of the diatom between the 20th and 30th day after transfer to the modified BSW-2 medium.

Results

Growth. The mean growth rates of *B. obtusa* at various combinations of temperature and daylength are shown in Table 4. Growth only occurred at temperatures below 18℃ except under a short daylength at 18℃. *B. obtusa* did not grow at 22℃. The maximum mean growth rate occurred at 14℃ under the 14 : 10 hr LD cycle. A similar mean growth rate occurred at 14℃ under the 10 : 14 hr LD cycle and at both daylengths at 10℃ (t-test; $P>0.05$). The mean growth rates at 5℃ and 18℃ were significantly different from the value at 14℃ under the 14 : 10 hr LD cycle ($P<0.05$). The mean growth rates were not significantly different between the long and short daylengths ($P>0.05$).

The mean growth rates of *B. obtusa* at different light intensities at the three levels of temperature are shown in Table 5. At each temperature there is no significant difference between means of different light intensities (one-way ANOVA; $P>0.05$).

The mean growth rates of four epiphytic diatoms at three different temperatures are shown in Table 6. The maximum mean growth rate occurred at 14℃ in *F. capucina* var. *capucina*, *Melosira* sp. and *Navicula* sp., and at 5℃ in *S. investiens*. These diatoms showed more rapid growth than *B. obtusa* under all conditions examined (cf. Table 5).

Viability. Text Figure 6 shows the growth of *B. obtusa* under three different illumina-

Table 5 Influence of temperature and light intensity at a 14 : 10 hr LD cycle on the growth of *Berkeleya obtusa*.

	Growth rate (divisions/day) ($\bar{X}\pm SD$; n=3)		
Temperature (℃)	Light intensity (lx) 1000	3000	5000
5	0.25±0.12	0.32±0.07[1)]	0.42±0.01[1)]
10	0.49±0.06	0.49±0.06	0.47±0.07
14	0.47±0.09	0.58±0.04	0.57±0.04

[1)] N=2.

Table 6 Influence of temperature on the growth of four diatoms observed on the colonial tube of *Berkeleya obtusa*[1].

Taxon	Growth rate (divisions/day) ($\bar{X}\pm SD$; n=3)		
	5 ℃	10℃	14℃
Fragilaria capucina var. *capucina*	0.59±0.15	0.90±0.07	1.19±0.16
Melosira sp.	0.40±0.13	0.77±0.23	1.07±0.04
Navicula sp.	0.90±0.20	1.10±0.35	1.65±0.40
Synedra investiens	1.42±0.07	1.22±0.17	1.16±0.06

[1] At a 14 : 10 hr LD cycle of light intensity of 3,000 lx.

tion conditions. The growth was the most rapid at 3,000 lx. Maximum cell numbers (5.3×10^4cells/ml) were observed in the 2-month-old culture. At 170 lx, slower growth was observed which continued to the end of the experiment. A cell-concentration of 3.7×10^4 cells/ml was observed in the 6-month-old culture. In complete darkness, the maximum cell (1.4×10^3 cells/ml) was observed in one-month-old culture. Thereafter cell-concentration decreased gradually.

Table 7 shows the survival of *B. obtusa* from prolonged exposure to three illumination conditions at 14℃. Cells illuminated at 3,000 lx for 6 months maintained viability, although the growth reached a maximum in the 2-month-old culture (Text Fig. 6). The cells turned to white after 6 months. Cells illuminated at 170 lx for 6 months also maintained viability. Cells exposed to the complete darkness for two months exhibited

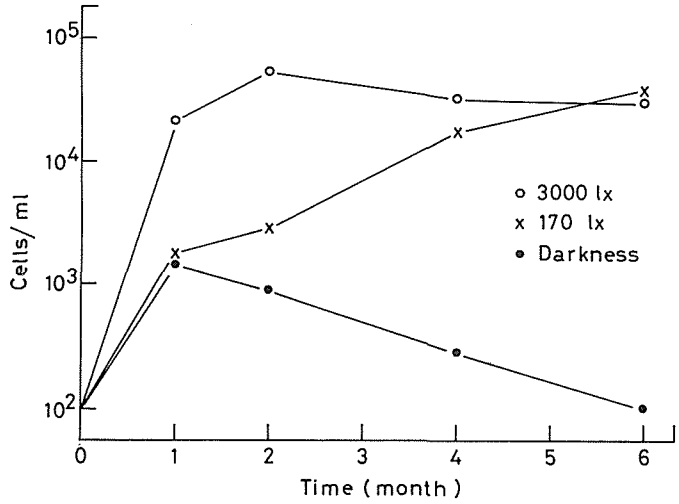


Fig. 6 Growth of *Berkeleya obtusa* under different illumination conditions. The light-dark cycle was 14 L : 10 D hrs.

Table 7 Survival of *Berkeleya obtusa* from prolonged exposure to three light conditions at 14℃.

Light condition	Duration of incubation (month)	Survival[1)]
3,000 lx and 14 : 10 hr LD cycle	6	3/3 [2)]
170 lx and 14 : 10 hr LD cycle	1	3/3
	2	3/3
	4	2/2
	6	3/3
Dark	1	3/3
	2	3/3
	4	2/3
	6	0/3

[1)] Judging from growth between 20 th and 30 th day after transferring into modified BSW-2 medium.

[2)] The numerator is the number of replicates in which growth was observed. The denominator is the total number of replicates.

viability. However, the viability declined in the cells which had exposed to the complete darkness for 4 months. In one of the three replicas, growth did not occur after transfer to the modified BSW-2 media. Cells exposed to complete darkness for 6 months did not grow at all after transfer to the modified BSW-2 media.

Discussion

A discrepancy between the temperature for rapid growth in the field and for the maximum growth rate in the laboratory has been reported hitherto (SMAYDA 1969, DURBIN 1974). DURBIN (1974) mentions that the disapperance of *Thalassiosira nordenskiöldii* in the spring is not determined only by rising temperature; some other factors or combination of factors are more important in causing its disappearance in natural waters around the optimal temperature for growth. Field observations show that *B. obtusa* is regarded as a cold water diatom (Chapter II). It has been shown that *B. obtusa* occurs mainly from autumn to mid spring and occurs sporadically from July to September (Chapter II). During both the peak and sporadic occurrences, the monthly mean water temperature at 13 : 00 was 2-12℃ and 17-21℃, respectively (MIZUNO 1984a). During the stage of decrease (March-April) following the main occurrence, the monthly mean water temperature was 3-7℃ at 13 : 00, and during the period of absence (May-June and October) temperatures were 9-17℃ and 15-17℃, respectively (MIZUNO 1984a). In culture experiments, however, *B. obtusa* showed a relatively low growth rate at 5℃, very low rate at 18℃ and no growth at 22℃, while at 10℃ and 14℃ it showed a relatively high growth rate. The results of the field and labora-

tory studies suggest that the mean water temperature during the peak occurrence is lower than the optimum for the growth and during the sporadic occurrences exceed the tolerance limit for the growth. On the other hand, the mean water temperatures during the absence of the taxon have been near or within the optimum for growth. Thus the seasonal occurrence of *B. obtusa* will not be determined by monthly mean seawater temperature.

The lower low water of spring tide occurs in the daytime from late March to early September (MIZUNO 1984a). Water temperature in tide pools rises up to 18℃ or more during the emersion in the daytime from March until early September (MIZUNO 1984a). The high water temperature during emersion might be one of causes why *B. obtusa* abruptly decreases in spring and disappears in summer and early autumn.

Some reports have shown that the growth rates of planktonic and benthic diatoms varied with the daylength (CASTENHOLZ 1964, PAASCHE 1968, ADMIRAAL 1977). The growth rate of the present diatom scarcely increased during the lengthening of the photoperiod.

The culture experiments have demonstrated that the growth rate of the present diatom is saturated below 1,000 lx. The light intensities for saturation of the growth rate in most of diatoms reported (see EPPLEY 1977) are higher than that found in *B. obtusa*. However, some reports show diatoms grow well at a low light intensities. The same maximum growth rate was obtained at 750 lx in *Chaetoceros aramatum* T. WEST (LEWIN and MACKAS 1972). A very low level of daily quantum irradiance, 0.6 $E \cdot m^{-2} day^{-1}$, was sufficient for three benthic diatoms to attain a growth rate equalling one half of their maximum growth rate (ADMIRAAL 1977). This quantam irradiance is near 500 lx using the following relation: 1 $\mu E \cdot m^{-2} s^{-1} = 50$ lx (LÜNING 1981). The light intensity of saturation for the growth rate of *B. obtusa* is lower than that of eulittoral seaweeds and is near that of sublittoral seaweeds (cf. LÜNING 1981). It is probable that *B. obtusa* may be considered a "shade plant", as are sublittoral seaweeds (LÜNING 1981).

Some workers believe that exposure to direct solar radiation is an important factor affecting the seasonal occurrence of littoral diatoms (ALEEM 1950, CASTENHOLZ 1963, HOPKINS 1964). Sublittoral seaweeds have less resistance to direct solar radiation than littoral seaweeds (BIEBL 1962). They were killed by exposure to direct sunlight (about 10^5 lx) for 2 hours (BIEBL 1956b). The lower low water of spring tide occurs in the daytime from late March to early September at Charatsunai shore (MIZUNO 1984a). If the present diatom is a shade plant as are sublittoral seaweeds, there is possibility that it is damaged by exposure to direct sunlight during the emersion of daytime.

The epiphytic diatom's fouling becomes heavy in the latter half of the period of occurrence of *B. obtusa* at the shore (Chapter II). Growth experiments show that this fouling seems to be due to the faster growth of epiphytic diatoms. This fouling probably influences nutrient uptake and light absorption of *B. obtusa*.

The survival of marine microalgae in darkness has been examined by several workers. *Rhizosolenia fragilissima* lost the viability within 28 days (IGNATIADES and SMAYDA

1970b), while several planktonic diatoms belonging to the genera *Asterionella, Chaetoceros* and *Thalassiosira* maintained the viability after 90 days (SMAYDA and MITCHELL-INNES 1974). *Phaeodactylum tricornutum* BOHLIN survived for 24 weeks (ANTIA and CHENG 1970).

The present diatom can survive for 4 months in continuous darkness. This diatom is absent from the shore for 6 months except for the sporadic occurrence in warm months (Chapter II). From the viability experiment, it is difficult to determine if this diatom survives in the dark zone until its next appearance. On the other hand, the present diatom when exposed to light intensities of 170 lx and 3,000 lx for 6 months, maintains a growth potential. In the sublittoral zone it is known that light intensity is greatly reduced, compared with the surface (ARUGA 1973, LÜNING 1981). The viability experiment shows that the present diatom can grow at 170 lx. It is likely that this diatom survives in the sublittoral zone until the next appearance.

2. Potential of the surface water of Charatsunai for the growth of *Berkeleya obtusa* and nutrient-enrichment experiments

I have shown that the seasonal occurrence of *Berkeleya obtusa* at Charatsunai shore will not be regulated solely by seawater temperature (Chapter III-1). The concentrations of nitrate, phosphate and silicate fluctuate seasonally in the seawater of Charatsunai shore. These nutrients are high from late autumn to early spring and poor in other seasons (MIZUNO 1984a). The field observations show that the main occurrence of *B. obtusa* is from late autumn to middle spring (Chapter II). Furthermore, the nutritional experiments show that *B. obtusa* requires nitrate, phosphate and silicate for the growth (MIZUNO 1983). From these studies, it is suggested that the occurrence of *B. obtusa* at the shore may be related to the concentrations of the three nutrients.

To examine the influence of nutrients on the seasonal occurrence of the algae or the growth potential of water, nutrient-enrichment experiments have been used (IGNATIADES and SMAYDA 1970b, RYTHER and DUNSTAN 1971, SCHELSKE and STOERMER 1971, SMAYDA, 1973, 1974, NISHIHAMA and IWASAKI 1974, NISHIHAMA 1975, UCHIDA 1981).

Material and Methods

An axenic clone of *B. obtusa* collected from Charatsunai in October 1978 was used in this experiment. The medium and condition for stock culture were described in Chapter III-1.

During four "seasons" surface seawater samples were collected at the Point-B (MIZUNO 1984a) of Charatsunai shore. Collection dates and nutrient concentrations are given in Table 8. The methods of nutrient analysis are described previously (MIZUNO 1984a). Five hundred milliliters of the seawater sample was filtered through a membrane filter immediately after collection. Fifty milliliters of the seawater for washing the stock

Table 8 Chemical analyses of the seawater samples used in the enrichment experiments.

Collection date	7/29/78	9/30/78	12/28/78	1/29/79	2/27/79	3/28/79	4/28/79
No_3-N μg at/liter	0.7	0.7	4.3	6.4	2.1	1.4	0.7
PO_4-P μg at/liter	0.3	0 [1)]	1.0	1.0	0.3	0.3	0.3
SiO_2-Si μg at/liter	2.1	4.3	10.7	19.2	7.8	8.9	0 [1)]

[1)] Zero indicates undetectable amounts with the analytical methods used.

culture and preculturing was poured in to a 100 m*l* polyethylene bottle and 450 m*l* of the seawater for the enrichment experiment was poured into a 500 m*l* polyethylene bottle. These seawaters were frozen at −15℃ until the experiment was carried out. After thawing in tepid water, the seawater was filtered through a sterile membrane filter and 10 m*l* aliquots were dispensed aseptically into sterile culture vessels. A 50 m*l* polycarbonate flask was used as a culture vessel in this experiment according to LEWIN (1966). The seawater was enriched in 9 different ways by adding a solution (0.2 m*l*) of each combination of nutrients (Table 9): (1) unenriched natural seawater (U); (2) U+Fe+B_{12} (E); (3) E+N; (4) E+P; (5) E+Si; (6) E+N+P; (7) E+N+Si; (8) E+P+Si; (9) E+N+P+Si (complete medium, ALL). The nutrients added and their final concentrations are shown in Table 9. Tris (hydroxymethyl) aminomethane was added to all enrichments as a buffer. Fe and Vitamin B_{12} were added to all enrichments except for the unenriched seawater (U) because *B. obtusa* requires these two nutrients for growth (MIZUNO 1983).

The preculture and enrichment experiments were carried out at 14℃ with a 14 : 10 hr LD cycle. The light intensity was 3,000 lx. These conditions were optimal for the growth of *B. obtusa* (Chapter III-1). The cells grown in stock culture were washed once with 5 m*l* of a sterile unenriched seawater (U) with the same seawater that was used in the nutrient enrichment experiments and were precultured in 10 m*l* of U. After 6 days, an inoculum of 50-200 cells/m*l* was added to each of the nine enrichments. Each enrichment was run in triplicate. The experiments were terminated after 10 days. Since this diatom excretes gelatinous material and forms a colony in the medium using natural seawater, two drops of concentrated HCl were added to the culture flasks and the flasks were heated to dissolve the

Table 9 Added nutrient and their final concentration.

$NaNO_3$	50 mg/liter of medium
K_2HPO_4	5 mg/liter
$Na_2SiO_3 \cdot 9H_2O$	10 mg/liter
Fe-EDTA	1 mg Fe/liter
Vitamin B_{12}	2 μg/liter
Tris (hydroxymethyl) aminomethane	1 g/liter
pH	7.8—8.0

gelatinous material. Cell number in 0.01 m*l* was counted using a ruled counting plate. The growth rate (divisions/day) for 10 days was calculated from the same equation described earlier (Chapter III-1).

Results

The mean growth rates obtained in the various enrichments are presented in Table 10. The difference between the mean growth rates of enrichments was significant in all months (one-way ANOVA ; $P<0.05$). The growth occurred in the unenriched seawater (U) of all months, but the mean growth rate was the lowest among the enrichments. The relative growth rate in U to the complete medium (ALL) varied with months and was the highest in December and the lowest in July. Since the significant difference was not observed between those in ALL of July, December, January, February and March ($P>0.05$), the difference between the mean growth rates in U of these months was statistically analyzed by the one -way ANOVA. The mean growth rates in U were significantly different between these months ($P<0.001$). The mean growth rate in U of December was higher than those of July, February and March ($P<0.01$). but was not significantly different from that of January ($P>0.05$).

The mean growth rate in U was significantly different from that in ALL (one-way ANOVA ; $P<0.05$). Supplement of some nutrients to U was necessary to obtain the same growth rate as ALL. In the nutrient-poor *July* water, the same growth rate as ALL

Table 10 Growth of *Berkeleya obtusa* in surface seawater collected from the Charatsunai shore at various months and enriched in various ways. The value shows the percentage of the mean growth rate (divisions/day) obtained in the complete (ALL) medium.

Enrichment	Collection date						
	7/29/78	9/30/78	12/28/78	1/29/79	2/27/79	3/28/79	4/28/79
Unenriched (U)	36^{***}[1)]	61^{***}	85^{*}	77^{**}	46^{***}	67^{**}	66^{***}
Enriched (E=U+Fe+B_{12})	54^{***}	70^{**}	98^{NS}	90^{NS}	78^{*}	64^{**}	77^{***}
E+N	64^{***}	76^{*}	93^{NS}	87^{*}	78^{*}	56^{***}	74^{***}
E+P	85^{NS}	89^{NS}	105^{NS}	108^{NS}	95^{NS}	95^{NS}	83^{**}
E+Si	62^{***}	89^{NS}	103^{NS}	97^{NS}	93^{NS}	74^{*}	83^{**}
E+N+P	85^{NS}	83^{NS}	110^{NS}	113^{*}	80^{NS}	97^{NS}	85^{**}
E+N+Si	67^{***}	80^{*}	113^{*}	95^{NS}	90^{NS}	74^{*}	89^{NS}
E+P+Si	82^{*}	100^{NS}	113^{NS}	110^{NS}	85^{NS}	97^{NS}	94^{NS}
E+N+P+Si (ALL)	100	100	100	100	100	100	100
	(0.39)[2)]	(0.46)	(0.40)	(0.39)	(0.41)	(0.39)	(0.47)

[1)] Comparison between actual mean growth rates in enrichment (n=3) and in ALL (n=3) by a one-way ANOVA. NS=$P>0.05$, *=$P<0.05$, **=$P<0.01$ and ***=$P<0.001$.

[2)] Actual mean growth rate (divisions/day) obtained in ALL medium.

occurred in E+P and E+N+P. Although the growth rate in E+P+Si was significantly different from that in ALL ($P<0.05$), it was a near value to those in E+P and E+N+P. Supplement of phosphate was effective in improving growth. In the nutrient-poor *September* water, the same growth rate as ALL occurred in E+P, E+N+P and E+P+Si. Supplement of phosphate was effective. The same growth rate as ALL was obtained in E+Si, even though phosphate was not detected in the analysis (Table 8). In the nutrient-rich *December* and *January* waters, the same growth rate as ALL was obtained by supplying with only Fe and vitamin B_{12}. Superaddition of other three nutrients effected a little, although the mean growth rates in E+N+Si of December and E+N+P of January were higher than that of ALL ($P<0.05$). In the nitrate-and phosphate-poor *February* water, the same growth rate as ALL occurred in E+P, E+N+P, E+N+Si and E+P+Si. The supplement of phosphate or silicate was effective. In the nitrate-and phosphate-poor *March* water, the same growth rate as ALL occurred in E+P, E+N+P and E+P+Si. The supplement of phosphate was effective. In the nutrient-poor *April* water, the same growth rate as ALL occurred in E+N+Si and E+P+Si. The supplement of silicate combining with phosphate or nitrate was effective.

Discussion

The seasonal fluctuation of nutrients is listed as one of factors governing the seasonal succession of phytoplankton (cf. GUILLARD and KILHAM 1977). Some investigators examined the influence of variation of water quality on the occurrence of a certain phytoplankton by means of nutrient enrichment (IGNATIADES and SMAYDA 1970b, SMAYDA 1973, UCHIDA 1981). IGNATIADES and SMAYDA (1970b) showed an inadequacy of trace metals in seawater appeared to be one of the factors preventing active growth of *Rhizosolenia fragilissima* BERGON during late fall and winter, and during late spring and early summer in Narragansett Bay. SMAYDA (1973) reported that low concentrations of nitrogen and silicon limited the growth of *Skeletonema costatum* (GREV.) CL. from mid-March through mid-June in Narragansett Bay. UCHIDA (1981) reported that phosphate was considered to limit the growth of *Prorocentrum micans* EHR. in Muroran harbor.

The present study has demonstrated that the potential of seawater in Charatsunai for the growth of *B. obtusa* varied seasonally. It was high in December and January and low in other months. The high potential was obtained in the seawater with high concentrations of nitrate, phosphate and silicate (Tables 8 and 10). *B. obtusa* occurs from late autumn to middle spring at Charatsunai and disappears in other seasons except for small sporadic occurrences (Chapter II). The period when the high potential of seawater for growth of *B. obtusa* approximately coincides with the occurrence period of the diatom at study site. The seasonal variation in the concentrations of these three nutrients likely affects the occurrence of the diatom.

The relative growth rates in U to ALL in December and January were higher than other

months, but were lower than ALL. The equivalent growth to ALL occurred by supplying U with only Fe and vitamin B_{12}. The deficiency of Fe and/or vitamin B_{12} seems to be a cause why the growth rate in U could not be obtained the same growth rate as ALL. In the seawaters of these months, the concentrations of nitrate, phosphate and silicate seem to be not deficient for growth, because superaddition of these three nutrients scarcely stimulated the growth rate. The deficient nutrients causing depression of growth in seawaters of other months are thought to be as follows: phosphate in July, September and March; phosphate (or silicate?) in February; silicate with nitrate or with phosphate in April. The deficiency of Fe and/or vitamin B_{12} might be another cause in July and February, because the mean growth rates between U and E were significantly different (one-way ANOVA; $P<0.05$).

The growth rate occurred in U of the *September* water in which phosphate was not detectable and the equivalent growth rate to ALL was obtained in E+Si (Tables 8 and 10). The growth occurred in U of the *April* water in which silicate was not detectable (Tables 8 and 10). According to the data of chemical analysis of the February water (Table 8) and the results obtained in enrichment experiment of July, September and March (Table 10), the deficient nutrient is expected to be phosphate and not silicate in the *February* water. However, the equivalent growth rate to ALL was observed in E+Si and E+N+Si as well as in enrichments with phosphate (Table 10). My previous study showed that *B. obtusa* could grow in phosphate-and silicate-free media after starvation for 7 days in phosphate-free medium and for 5 days in silicate-free medium, respectively (MIZUNO 1983). These might be some of causes why the growth occurred in the *September* and *April* waters, and a high growth rate was obtained in some enrichments without supplement of phosphate in September and February.

The results of enrichment experiments suggest that the seasonal variation in water quality is one of factors governing the seasonal occurrence of *B. obtusa*.

3. Influence of daily short term exposure to high pH on the growth of *Berkeleya obtusa*

The pH value of the seawater usually ranges from 7.5 to 8.4 (SVERDRUP *et al.* 1942, KOKUBO 1962, ARUGA 1973). However, as a result of photosynthesis and respiration, the pH of water in tide pools may rise to more than 9 or fall to less than 7.5 (ATKINS 1919-22, PYEFINCH 1943, ALEEM 1950, EDELSTEIN and MCLACHLAN 1975). At Charatsunai shore, the pH of seawater in tide pools fell to nearly 7.5 when emersion occurred in the nighttime, while the pH rose to 9.0 or more when emersion occurred in the daytime (MIZUNO 1984a). The measurements of pH (MIZUNO 1984a) and the field observations of *B. obtusa* (Chapter II) suggest that the rise of pH might be related to the disappearance of *B. obtusa* from the study site. Furthermore, a laboratory experiment showed that *B. obtusa* grew well from pH 7.58 to pH 8.25, while growth decreased at a pH of more than 8.60 (MIZUNO 1983). The extremely high pH in tide pools is maintained for many hours, but not for an entire day,

as the tide pools are submerged by the returning tide.

The influence of daily short term exposure to high pH on the growth of *B. obtusa* has been examined in this experiment.

Material and Methods

An axenic clone of *B. obtusa* collected from Charatsunai in October 1977 was used in this experiment. The medium and condition for stock culture were described in Chapter III-1. A modified BSW-2 (Table 3) was used as the experimental culture medium. To obtain the high pH medium, a higher pH medium was prepared and then adjusted with 1 N HCl to the desired pH. A glass tube (1.5 cm×12 cm) closed at the bottom by a membrane filter and a petri dish (6 cm×13 cm) were prepared as culture vessels (Text Fig. 7A). The pore size of the membrane filter was 3 μm in diameter through which the cells of the present

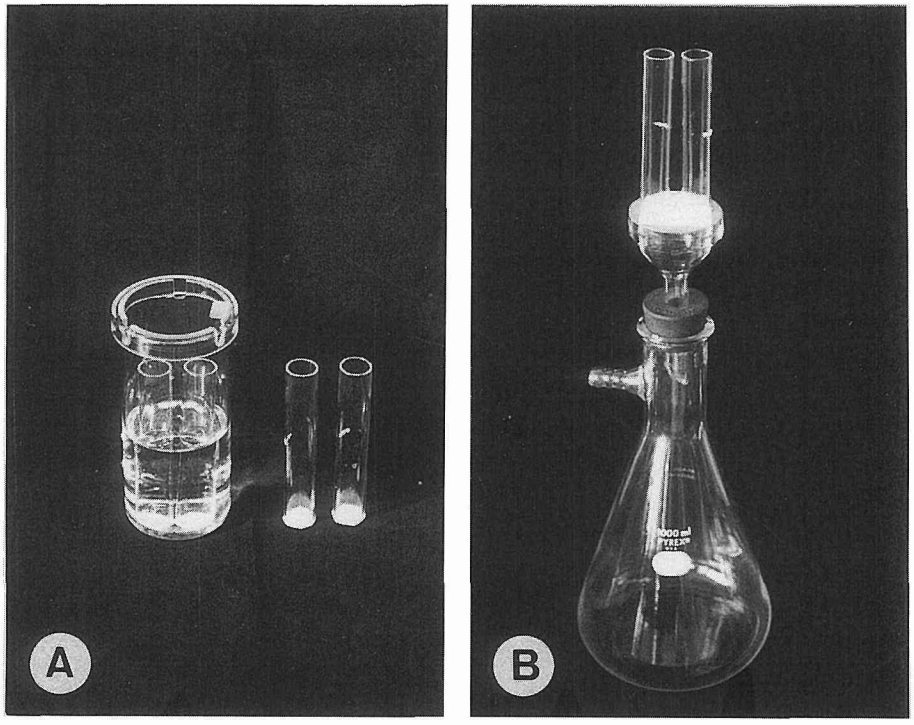


Fig. 7 Culture vessels and draining apparatus used for the *B. obtusa* experiment on the influence of daily exposure to short term and extreme pH or salinity. A: Glass tubes for transferring the diatom cells from normal medium to extreme pH or salinity medium and vice versa. The tubes were closed at the bottom by a membrane filter (pore size of 3 μm). The diatom cells were inoculated into the tubes (right). Culture vessel containing medium and the tubes (left); B: Aspirator draining medium from the tube. The medium was drained through the membrane filter at the bottom of the tube.

diatom could not pass. The two glass tubes were set in a petri dish containing 100 m*l* of the medium (Text Fig. 7A). The cells was inoculated into the tube. The tubes containing the cells were transferred daily from the petri dish containing the medium with normal pH (8.0) to another petri dish containing the medium with high pH (8.9-9.0). They were returned to the petri dish containing the medium with normal pH after 3, 6 and 9 hours, respectively. The transfer to high pH was undertaken as follows: (1) drain the normal pH medium from the test tube with the aspirator (Text Fig. 7B); (2) wash with 5 m*l* of the fresh high pH medium; (3) drain; (4) add 10 m*l* of the fresh high pH medium; (5) transfer to the petri dish containing the high pH medium. After exposure to high pH, the test tube and contents were treated as follows: (1) drain the high pH medium from the test tube with the aspirator; (2) wash with 5 m*l* of the fresh normal pH medium; (3) drain; (4) add 10 m*l* of the fresh normal pH medium; (5) return to the petri dish containing the normal pH medium. The step of washing during the processes of transfer (2 and 3) was omitted in the second experiment. In the treatments of exposures to high and normal pH for 24 hours, the daily draining was carried out the same times as in other treatments. The daily transfer was done in light phase. The experimental condition was set at 14℃ during 14 : 10 hr LD cycle of 4,000 lx. The experiment was carried out under a xenic condition. An inoculum of 14,000 cells/tube was used in the first experiment and 19,000 cells/tube in the second experiment. After 7 days, the cell number was measured with a hemacytometer. The growth rate (divisions/day) for 7 days was calculated from the same equation described in Chapter III-1.

Results

The mean growth rates of *B. obtusa* under different durations of daily exposure to high pH are shown in Table 11. The mean growth rates in control and the degree of depression

Table 11 Influence of daily exposure to high pH (pH 8.9—9.0) on the growth of *Berkeleya obtusa*[1].

Treatment	Exp. 1	Exp. 2
Control (pH 8.0 for 24 hr)	100% (0.24)[2]	100% (0.40)
High pH for 3 hr	92[NS3]	93[NS]
High pH for 6 hr	42[NS]	75*
High pH for 9 hr	25*	63**
High pH for 24 hr	4*	53**

[1] The value shows the percentage of the mean growth rate (divisionsKday) obtained in control.

[2] Actual mean growth rate (divisions/day) (n=2) obtained in control.

[3] Comparison between actual mean growth rates in treatment (n=2) and in control by a one-way ANOVA. NS=$P>0.05$, *=$P<0.05$ and **=$P<0.01$.

of growth rate due to exposure to high pH were different between the first and second experiments. These may be due to the different protocols used in the two experiments. The step of washing was omitted in the second experiment. Additional draining and washing in the first experiment might have caused the lower growth rates. The highest mean growth rate was obtained in control (pH 8.0 for 24 hours). The mean growth rate was declined with increasing length of exposure to high pH. The lowest one was obtained in the treatment of exposure to high pH for 24 hours. The mean growth rates were significantly different between treatments in both experiments (one-way ANOVA; $P<0.05$). In the first experiment, the mean growth rates in the treatments of exposure for 3 and 6 hours were not significantly different from that of control ($P>0.05$), but those in the treatments of exposure for 9 and 24 hours were significantly different from that of control ($P<0.05$). In the second experiment, the mean growth rate in the treatment of exposure for 3 hours was not significantly different from that of control ($P>0.05$), but those in the treatments of exposure for 6, 9 and 24 hours were significantly different from that of control ($P<0.05$).

Discussion

Although the pH value of the seawater at the Charatsunai study site is 8.0-8.5, the pH value of stagnant water in the clefts or the seawater in tide pools falls near pH 7.5 when the emersion occurs in the nighttime (MIZUNO 1984a). The previous study (MIZUNO 1983) shows that the optimal pH value for the growth of *B. obtusa* ranges from pH 7.58 to pH 8.25. The low pH value observed at the emersion in the nighttime is thus unlikely to affect the growth of *B. obtusa*.

On the other hand, the pH value rises near pH 9.0 when the emersion occurs in the daytime (MIZUNO 1984a). This pH value exceeds the optimal pH value for the growth and may continue until the shore and tide pool are submerged by the returning tide. The times and duration of emersion for 5 days at each spring tide have been estimated at different levels in the littoral zone of Charatsunai (MIZUNO 1984a). According to this estimate an exposure duration of 3 hours occurs in the daytime from April to July at the +10 cm level of the tidal datum line (the tidal datum line is near the level of the lowest low water of spring tide). At the +40 cm level, exposure durations of 3-5 hours and 1-4 hours in the daytime frequently occur from March to July and from August to September, respectively. At the +60 cm level, durations of 5-7 hours and 3-6 hours frequently occur from March to July and from August to September, respectively. Additionally, a duration of 3 hours occurs in the daytime in February and October. Emersion occurs in the daytime throughout the year at the 80 cm level. However, the duration is short (*ca.* 4 hours) from November to February. Durations of 6-8 hours and 3-7 hours frequently occur from March to July and from August to October, respectively. At the +160 cm level emersion continues all day from February to April and in September and a duration of more than 21 hours occurs in other months.

The results of the culture experiment show that the daily exposure to high pH for 3 hours rarely suppresses the growth of *B. obtusa*, while the daily exposure for 6 hours or more does. Judging from the results of the field measurements and culture experiments, high pH probably does not affect the growth of *B. obtusa* at the level of +10 cm. At the level of +40 cm, high pH probably affects the growth from March to July. However, high pH does not affect the growth from August to September, because the duration of emersion becomes short. At the levels of +60 cm and +80 cm, high pH probably affects the growth from March to September. At the level of +160 cm, high pH probably affects the growth throughout the year.

B. obtusa disappears from the shore from May to October (Chapter II). Rising of pH during the emersion of the daytime is thought to be one of factors causing the disappearance of *B. obtusa* during warm months. However, some other factor(s) seems to govern the disappearance at the level of +10 cm. *B. obtusa* does not occur at the level of +80 cm during winter, although the exposure calculations and culture experiments show that the pH at this level has little influence on the growth. Some other factor(s) seems to prevent the winter occurrence at this level. At the level of +160 cm, *B. obtusa* can not grow throughout the year because of a long duration of exposure to high pH.

The pH seems to be one of factors governing the seasonal occurrence and vertical distribution of *B. obtusa*.

4. Influence of daily short term exposure to high and low salinity on the growth of *Berkeleya obtusa*

The salinity of seawater in the tide pools or their clefts at lower low water of the spring tide occasionally rises to 41‰ in the cold season or falls to 14‰ in the warm season at Charatsunai shore. Usually it ranges from 31-34‰ (MIZUNO 1984a) and differs little from the salinity of the coastal water in Uchiura Bay (Volcano Bay) (cf. OHTANI *et al.* 1971a, 1971b).

A laboratory experiment (MIZUNO 1983) showed that *B. obtusa* grew well in the range from 23.9 to 33.0‰, while growth was reduced at 19.1‰ and at 42.0‰. Judging from the results of the laboratory experiment, the high or low salinity observed at the shore will suppress the growth of *B. obtusa*. Extreme high or low salinities occur for hours, but not for a full day, because the shore is resubmerged by returning tide.

The influence of daily short term exposure to high and low salinity on the growth of *B. obtusa* has been examined in this experiment.

Material and Methods

An axenic clone of *B. obtusa* collected from Charatsunai in October 1977 was used in this experiment. The medium and condition for stock culture are described in Chapter III-1. Sea water which had been concentrated by heating at 70-80℃ or diluted with distilled

water was enriched in the same way as a modified BSW-2 (Table 3). By means of this procedure a medium with a high or low salinity was obtained. The same culture vessels were used as in the experiment of daily exposure to high pH (Chapter III-3). Two glass tubes were set in a petri dish containing 100 m*l* of medium (Text Fig. 7A). The material was inoculated into the tube. The tubes containing material were transferred daily from the petri dish containing the medium with normal salinity (30-31‰ of S) to another petri dish containing the medium with high (39-41‰) or low (14‰) salinity. They were returned to the petri dish containing the medium with normal salinity after 1 and 3 hours, respectively. The transfer to high or low salinity was undertaken as follows: (1) drain the normal salinity medium in the tube with the aspirator (Text Fig. 7B); (2) add 10 m*l* of the fresh high or low salinity medium; (3) transfer to the culture vessel containing high or low salinity medium. After exposure to high or low salinity, the tubes were treated as follows: (1) drain the high or low salinity medium in the tube with the aspirator; (2) add 10 m*l* of the fresh normal salinity medium; (3) return to the culture vessel containing normal salinity medium.

In the treatments of exposures to high, normal and low salinity for 24 hours, the daily draining was carried out the same times as in other treatments. The daily transfer and return were done in light phase. The experiment was done under the condition of 14℃ durng 14 : 10 hr LD cycle of 4,000 lx. The experiment was carried out under the xenic condition. Inocula were 33,000 cells/tube in each experiment. After 8 days, cell number was obtained with a hemacytometer. The growth rate (divisions/day) for 8 days was calculated from the same equation described in Chapter III-1.

Results

The mean growth rates under different durations of daily exposure to high or low salinity are shown in Table 12. No growth of *B. obtusa* occurred in all treatments of daily exposure to low salinity in both experiments. The difference between mean growth rates of control and treatments of exposure to high salinity in the first experiment was significant (one-way ANOVA; $P<0.01$) and mean growth rates of all treatments of high salinity were sigificantly smaller than that of control ($P<0.01$). However, mean growth rates between different durations of exposure were not significantly different ($P>0.05$). The tendency that exposure to high salinity might suppress to growth was observed in the second experiment, although the significant difference was not observed between mean growth rates of control and treatment of high salinity ($0.1>P>0.05$).

Discussion

Influence of salinity on the survival of many marine macroalgae has been examined (BIEBL 1962, 1967, GESSNER 1969). Most intertidal algae can survive in a concentration range of 0.1 to 3.0 times that of seawater. Algae from tide pools or near low water mark,

Table 12 Influence of daily exposure to high and low salinity on the growth of *Berkeleya obtusa*[1].

Treatment	Exp. 1	Exp. 2
Control (30-31‰ of salinity for 24 hr)	100% (0.27)[2]	100% (0.29)
High salinity (39-41‰ of S) for 1 hr	41**[3]	59
High salinity for 3 hr	33***	59
High salinity for 24 hr	44**	83
Low salinity (14‰ of S) for 1 hr	NG[4]	NG
Low salinity for 3 hr	NG	NG
Low salinity for 24 hr	NG	NG

[1] The value shows the percentage of the mean growth rate (divisions/day) obtained in control.

[2] Actual mean growth rate (divisions/day) (n=2) obtained in control.

[3] Comparison between actual mean growth rates in treatment of exposure to high salinity (n=2) and in control by a one-way ANOVA. **=$P<0.01$ and ***=$P<0.001$.

[4] No positive growth.

however, exhibit narrow osmotic ranges (BIEBL 1962, 1967). The sublittoral brown alga *Dictyopteris membranacea* (STACKHOUSE) BATTERS is very sensitive to low salinity. One-minute exposure to distilled water causes total break down of photosynthesis and respiration of this alga and its photosynthetic mechanisms is irreversibly damaged by the exposure to salinity of lower 20‰ for 30 minutes (GESSNER 1969). Photosynthesis of the littoral brown alga *Fucus virsoides* J. AGARDH, however, is not affected by distilled water (GESSNER 1969).

Exposure to high (39-41‰) salinity for only one hour per day reduces the growth rate of *B. obtusa*. At low salinity (14‰) this diatom does not grow. These results show that *B. obtusa* is sensitive to high or low salinity as are sublittoral macroalgae, although it occurs in the littoral zone.

The salinity of seawater during emersion occasionally falls to 14‰ during rain in warm season at Charatsunai (MIZUNO 1984a). There is a probability that *B. obtusa* is adversely affected by the low salinity when it occurs in the littoral zone in warm season. A low salinity at emersion might be one of factors suppressing the occurrence of the diatom in warm season. In cold season, the salinity of seawater during emersion rises as a result of freezing-out of salt during ice formation. A high salinity of near 40‰ has been observed in a pool which is located at the upper littoral zone (MIZUNO 1984a). There is a probability that *B. obtusa* is affected in the pools in which high salinity is occasionally observed. For example, the diatom will be unable to grow in the Pool-4 at the level of +160 cm in cold season. A high salinity at emersion in cold season might be one of factors determining upper limit of growing zone of the diatom.

5. Survival of *Berkeleya obtusa* under various desiccating conditions

Since intertidal algae are periodically exposed at low tide, they must be able to withstand desiccation. The ability of certain intertidal algae to resist desiccation over a period of days has been recognized (MUENSCHER 1915). On the other hand, sublittoral algae are less capable of tolerating desiccation (MUENSCHER 1915, BIEBL 1956a, 1962, 1967, ZANEVELD 1969). MCINTIRE and WULFF (1969) and WULFF and MCINTIRE (1972) using a laboratory model ecosystem showed that the duration of exposure to desiccation affected the vertical distribution of marine benthic diatoms. ALEEM (1950) and HOPKINS (1964) pointed out that desiccation by solar radiation and drying winds was the most important of all factors affecting the seasonal occurrence of littoral diatoms.

The main occurrence of *B. obtusa* begins in November or December and ends in March or April (Chapter II). The lower low water of spring tides occurs in the nighttime from late September to early March and in the daytime from late March to early September (MIZUNO 1984a). The period of occurrence of *B. obtusa* approximately corresponds to the period when the lower low water of spring tide occurs at nighttime. During this period the shore is exposed to the air with a low temperature and low evaporation rates. During the period of the decrease and disappearance of *B. obtusa* the shore is exposed to air with a high temperature and high evaporation rates (cf. MIZUNO 1984a). Thus the influence of exposure to various desiccating conditions on the survival of *B. obtusa* has been examined.

Material and Methods

An axenic clone of *B. obtusa* collected from Charatsunai in October 1977 was used in this experiment. The medium and condition for stock culture are described in Chapter III-1. The material used in these experiments was inoculated in a 50 m*l* Erlenmeyer flask containing 20 m*l* of a modified BSW-2 medium (Table 3) and cultured under the same condition as the stock culture. After 12-17 days, one drop of the diatom suspension (*ca.* 5,000-12,000 cells/one drop) was placed on a dry glass filters of 1 cm^2 which were precooled to the experimental temperatures (*i. e.* −13, +5, +14, and +22℃). The culture medium was absorbed by the glass filters. A petri dish (8 cm×2 cm) containing four glass filters inoculated with diatom cells was exposed to several temperatures and evaporation rates (Table 13). The cover of the petri dish was removed except for the no evaporation condition. In this latter case the petri dish contained a small 1 m*l* vessel full of distilled water was placed beside the filter. The petri dish was then covered with PARAFILM (American Can Co.) to prevent evaporation. The experimental material was exposed to temperatures of −13, 5, 14 and 22℃. At 5, 14 and 22℃ the material was illuminated with cool white fluorescent lamps (1,000-3,000 lx). The material at −13℃ was kept in the dark. The evaporation rates were measured using the method described by MIZUNO (1984a). Exposure durations were 0.25, 0.5, 1, 3, 5 and 7 hours. After exposure to the various experimen-

tal conditions each glass filter with the material was transferred into a 100 m*l* Erlenmeyer flask containing 50 m*l* of a modified BSW-2 medium which had been precooled to the appropriate temperature. In the case of cells exposed to −13℃, the medium was precooled to 0℃. The flasks were then incubated at 14℃ on a 14 : 10 hr LD cycle at 3,000 lx. After 20 days, survival was measured by presence and absence of brown diatom colonies. The experiment was carried out under the xenic condition.

Table 13 Survival of *Berkeleya obtusa* under various desiccating conditions.

Temperature (℃)	Duration of exposure (hr)	Evaporation rate[1] (g/28 cm²/hr)		
		0	0.16±0.02	
−13	1	—[2]	11/12[3]	
	3	—	5/12	
	5	—	5/12	
	7	4/4	1/12	
		0	0.24±0.03	0.32±0.06
5	0.5	—	—	4/4
	1	—	4/4	4/4
	2	—	—	4/4
	3	—	4/4	0/4
	5	—	1/4	—
	7	4/4	0/4	—
		0	0.14±0.01	0.66±0.14
14	0.5	—	—	4/4
	1	—	8/8	1/4
	2	—	—	0/4
	3	—	1/8	0/4
	5	—	1/8	—
	7	4/4	0/8	—
		0	0.10±0.01	0.52±0.13
22	0.25	—	—	4/4
	0.5	—	4/4	3/4
	1	—	4/4	0/4
	2	—	—	0/4
	3	—	0/4	—
	5	—	0/4	—
	7	4/4	—	—

[1] $\bar{X}\pm SD$; number of measurements=3 or 4.

[2] Not examined.

[3] The numerator is the number of fiilters on which brown colonies of *B. obtusa* were observed on the 20 th day following transfer into a modified BSW-2 medium. The denominator is the total number of filters.

Results

The survivals at different temperatures and evaporation rates of water are shown in Table 13. Under the conditions of no evaporation, the diatom survived for at least 7 hours at all temperatures examined. The length of survival period decreased with increasing the evaporation rate at all temperatures. Most of the diatoms died within 3 hours at 14℃ at 0.14 g/28 cm²/hr, and none survived the 3 hour exposure at 22℃ at 0.10 g/28 cm²/hr, while some survived for 5 and 3 hours at −13℃ at 0.16 g/28 cm²/hr, and 5℃ at 0.24 g/28 cm²/hr, respectively. With high evaporation rates at 14℃ and 22℃, most or all of the diatoms died within one hour.

Discussion

MUENSCHER (1915) found that some littoral macroalgae survived after 1 or 2 days' air-drying, but sublittoral macroalgae died after only one hour exposure. BIEBL (1956a, 1962, 1967) reported that algae of the intertidal zone tolerated desiccation for 14 hours in air of 83-86% relative humidity, while sublittoral algae succumbed even in very moist air

Table 14 The monthly means of air temperature (℃) and evaporation rate (g/28 cm²/hr) in the nighttime from September to March and in the daytime from March to September.[1]

Month		Air temperature[2] (℃)	Evaporation rate[3] (g/28 cm²/hr)
September	daytime	22.1～ 24.1	0.74
	nighttime	17.2	0.15
October	nighttime	10.6～ 12.9	0.23
November	nighttime	5.3～ 5.4	0.10～0.13
December	nighttime	0.7	0.14
January	nighttime	−3.8～− 1.8	0.13～0.21
February	nighttime	−5.9～− 3.1	0.17～0.18
March	nighttime	0.1～ 0.3	0.11～0.13
	daytime	6.5～ 8.4	0.38～0.54
April	daytime	9.6～ 13.4	0.39～0.40
May	daytime	13.3～ 15.5	0.31
June	daytime	19.6～ 23.0	0.50
July	daytime	21.0～ 29.4	0.66
August	daytime	29.3	0.88

[1] Data from MIZUNO (1984a).
[2] Measured at 13 : 00 for daytime and at 20 : 00 for nighttime.
[3] Measured from 10 : 00 to 13 : 00 for daytime and for 2 or 3 hours just after sunset for nighttime.

(98.9% relative humidity). The present results show that *B. obtusa* is also sensitive to desiccation.

The lower low water of the spring tide occurs in the nighttime from late Septemger to early March at Charatsunai (MIZUNO 1984a). During this period, especially from November to February, the shore is exposed to air with a low temperature and a low evaporation rate during emersion (Table 14). On the other hand, the lower low water of the spring tide occurs in the daytime from late March to early September. During this period, the shore is exposed to air with a high temperature and a high evaporation rate during emersion (Table 14). The field measurements and the laboratory experiments suggest that *B. obtusa*: 1) will be able to survive for 5 hours under the condition of the emersion from November to February, 2) will die within 3 hours under the condition of the emersion from March to October, and 3) will die within one hour from June to September (cf. Tables 13 and 14). *B. obtusa* produces more gelatinous material in the field than in culture. Thus it should be able to survive for a longer time in the field because the richer gelatinous material seems to absorb and retain larger amounts of water.

The time and duration of emersion for 5 days at each spring tide were estimated for different levels in tidal height (MIZUNO 1984a). The +10 cm level of the tidal datum line (this line is near the lowest low water of spring tide) emerges in the nighttime for less than 4 hours from November to February and in the daytime for 2-3 hours from April to June. This level is always submerged in March and from August to October. There is a possibility that *B. obtusa* cannot survive the emersion in June at this level, while it can survive the emersions of other months.

The +40 cm level frequently emerges in the nighttime for 3-6 hours from October to March and in the daytime for 3-5 hours from March to August. In September this level emerges in the daytime for 1-2 hours and in the nighttime for 1-3 hours. There is a possibility that *B. obtusa* can survive November to February at the +40 cm level, while it cannot survive the emersion from March to October.

The +60 cm level frequently emerges during the nighttime for 5-8 hours from November to March, for less than 3 hours in April and August, and 3-6 hours from September to October. This level is always submerged in the nighttime from May to July. This level frequently emerges in the daytime for 5-7 hours from March to August, for 2-5 hours from September to October, and for less than 4 hours in February. This level is always submerged in the daytime from November to January. In the zone above +60 cm, a longer duration of emersion occurs than at the +60 cm level. There is a possibility that *B. obtusa* cannot survive emersion at +60 cm or above throughout the year.

Although the duration of the emersion period from November to February at +60 cm exceeds the duration of survival obtained in the laboratory experiment, *B. obtusa* appears at +60 cm of the shore. As described above, the difference in thickness of gelatinous material between the field and the laboratory experiment may partially explain the discrep-

ancy between the results of the field observations and the laboratory experiment.

The desiccation during the emersion seems to be one of important factors governing the seasonal occurrence and the vertical distribution of *B. obtusa* at Charatsunai.

6. Influence of the extracellular products of macroalgae on the growth of *Berkeleya obtusa*

Many algae release substances which inhibit or stimulate the growth of themselves or other species (see HELLEBUST 1974). Yellow extracellular phenolic substances produced by *Fucus vesiculosus* L. are inhibitory to the growth of some unicellular algae including a diatom (MCLACHLAN and CRAIGIE 1964). Water from *Ralfsia verrucosa* pools is toxic and affects animals (CONOVER and SIEBURTH 1966). It is known that phytoflagellates excrete diatom-inhibitory substances (PRATT 1966, UCHIDA 1977, 1981). Conversely, extracts of yellow material from *Ascophyllum nodosum* (L.) LEJOLIS stimulated bacterial respiration (YENTSCH and REICHERT 1962). *Enteromorpha linza* (L.) J. AGARDH liberates organic compounds which stimulate the growth of itself and other species (BERGLUND 1969).

Many macroalgae grow in the same zone of *B. obtusa* at the Charatsunai study site (Chapter II). At low tide, the seawater in shallow tide pools are separated from the open sea. Extracellular substances from macroalgae can accumulate in the pools at that time and may influence the growth of *B. obtusa* which co-occurs in the pools with the macroalgae.

Winter is the season of the main occurrence of *B. obtusa* and spring is the period of decreasing abundance (Chapter II). The release rate of extracellular products varies with external conditions and with physiological change in the plants themselves (CRAIGIE and MCLACHLAN 1964, KHAILOV and BURLAKOVA 1969). Since the emersion at the spring tide occurs in the nighttime in winter and in the daytime in spring, the shore environment at the emersion is different in winter and spring (MIZUNO 1984a). Furthermore, the composition of macroalgae at the shore is different in winter and spring (Chapter II).

The influence of extracellular substances produced by various macroalgae under the conditions of the emersions of winter and spring on the growth of *B. obtusa* has been examined in this study.

Materials and Methods

The standing crops of macroalgae in the area where the ecological observation of *B. obtusa* were conducted are reported in the Chapter II. Healthy macroalgae and the higher palnt *Phyllospadix iwatensis* MAKINO whose biomass exceeded 20 g of fresh weight per 10 quadrats in winter (January-February) or in spring (March-April) were collected from Charatsunai study site when they were submerged. They were transported immediately to the laboratory in a plastic bucket full of natural seawater. The plants were cleaned by removing epiphytes and small animals, washed with natural seawater, and lightly blotted.

A 300 m*l* Erlenmeyer flask containing 100 m*l* of filtered and aged natural seawater was

precooled to 0℃ or 14℃ before the experiment. The seawater was buffered with tris (hydroxymethly) aminomethane (100 mg/100 m*l* of seawater) and adjusted to the desired pH with 1 N hydrochloric acid. Ten g (fresh weight) of healthy plant material was inoculated into the flask. An intact or cut plant was used. The macroalgae and higher plant material were incubated under the following conditions: (1) 0℃, dark and pH 7.8-8.0; (2) 14℃, 5,000 lx (provided by cool white fluorescent lamps) and pH 8.9-9.0. Condition (1) and (2) are mimic the environmental conditions at the emersion period of winter and spring, respectively (MIZUNO 1984a).

The macroalgae and the higher plant which occurred abundantly from winter to spring were incubated at both 0 and 14℃. Those plants were *Kornmannia zostericola* (TILD.) BLIDING, *Ulva pertusa* KJELLMAN, *Colpomenia bullosa* (SAUND.) YAMADA, *Sargassum confusum* C. AGARDH, *Neorhodomela aculeata* (PEREST.) MASUDA, *Palmaria palmata* (L.) O. KUNTZE and *Phyllospadix iwatensis*. *Sargassum thunbergii* (MERT.) O. KUNTZE which is less abundant from winter to spring was incubated under both conditions because this alga was the substrate for *B. obtusa* (Ccapter II). Plants such as *Monostroma angicava* KJELLMAN, *Alaria crassifolia* KJELLMAN, *Analipus japonicus* (HARV.) WYNNE, *Laminaria japonica* ARESCHOUG, *Scytosiphon lomentaria* (LYNGB.) LINK and *Neodilsea yendoana* TOKIDA were incubated at 14℃ only, because those algae were less abundant in winter.

After 3 hours of incubation, the seawater conditioned by a macroalga or the higher plant was filtered through a glass filter. For estimating the amount of extracellular yellowish substances, optical densities of the conditioned seawater samples were determined at 275 nm by Hitach 124 spectrophotometer (CRAIGIE and MCLACHLAN 1964). Filtered seawater with tris (added 100 mg/100 m*l* of seawater) was used as the blank. The conditioned seawater was enriched by adding solution of nutrients. This enriched seawater medium is different from a modified BSW-2 (Table 3) in a final concentrations of 900 mg of tris and 900 m*l* of seawater per 1 *l* medium. Ten m*l* of the medium was sterilized through a sterile membrane filter (pore size of 0.22 μm) and was poured into a sterile screw-cap test tube (1.8 cm×13.5 cm). Three test tubes per one routine were supplied.

An axenic clone of *B. obtusa* collected from Charatsunai in October 1977 was used in this experiment. The medium and condition for stock culture are described in Chapter III-1. Cells in the exponential phase were inoculated into the conditioned and unconditioned media. Inocula of 100-1,700 cells/m*l* were used. The assay cultures were incubated for 9-16 days under the same condition as that of stock culture. The incubations were terminated by adding 2 drops of concentrated hydrochloric acid and heating. This treatment was necessary for dissolving the gelatinous materials embedding the cells. The cell number was counted using a hemacytometer. The growth rate (divisions/day) for 9-16 days was calculated with the same equation described earlier (Chapter III-1).

Table 15 Growth of *Berkeleya obtusa* in the media conditioned by various macroalgae[1].

Species used to condition the growth medium	Exp. No. (date)	Relative growth rate of *B. obtusa* in the media conditioned at: 0℃, dark, pH 8	14℃, 5,000 lx, pH 9
Chlorophyceae			
Kornmannia zostericola	1 (3/25/78)	97	78*[2]
	2 (3/25)	no growth	77*
	3 (4/6)	106	81
	4 (4/6)	108*	65*
Monostroma angicava	1 (3/31)	——[3]	81*
	2 (3/31)	——	73*
Ulve pertusa	1 (2/16)	96	91
	2 (2/19)	95	95
	3 (6/20)	94	94
Phaeophyceae			
Alaria crassifolia	1 (4/13)	——	42*
	2 (4/13)	——	16*
	3 (6/29)	——	80*
Analipus japonicus	1 (4/1)	——	102
	2 (4/8)	——	76*
	3 (4/8)	——	89
Colpomenia bullosa	1 (2/3)	100	100
	2 (2/15)	108	117
	3 (3/3)	104	110
Laminaria japonica	1 (4/14)	——	19*
	2 (4/14)	——	25*
Sargassum confusum	1 (2/9)	89	107
	2 (2/10)	64*	57*
	3 (3/29)	49*	70*
	4 (3/29)	43*	13*
	5 (4/15)	94	90
	6 (4/15)	81*	68*
	7 (5/1)	95	75*
	8 (5/1)	92	95
S. thunbergii	1 (2/11)	92	78*
	2 (2/13)	100	82*
	3 (6/22)	96	79*
Scytosiphon lomentaria	1 (4/12)	——	69*
	2 (4/12)	——	75*
Rhodophyceae			
Neodilsea yendoana	1 (4/11)	——	59*
	2 (4/11)	——	69*
Neorhodomela aculeata	1 (3/27)	98	57*
	2 (4/7)	100	92
	3 (4/7)	100	86*
Palmaria palmata	1 (2/25)	100	98
	2 (2/27)	97	94
Angiosperm			
Phyllospadix iwatensis	1 (5/11)	94	94
	2 (5/11)	96	94
	3 (6/22)	98	102

[1] The value shows the percentage of the mean growth rate (divisions/day) obtained in the unconditioned medium.

[2] Comparison between the mean growth rates in conditioned (n=3) and in unconditioned media (n=3) by t-test. *=significantly different at $P=0.05$.

[3] Not examined.

Results

The relative growth rates of *B. obtusa* in the media conditioned by 14 species are shown in Table 15. Differences between the results of the replicated experiments for each species were rarely observed except for *S. confusum*. Considerable variation in the results was observed between the experiments with *S. confusum*. The growth of *B. obtusa* was not reduced in media conditioned at 0℃ and at 14℃ in three of eight experiments. The growth was depressed in media at 0℃ and 14℃ in four and five of eight experiments, respectively. The results of the *S. confusum* experiments are excluded from the following description and described in discussion because of the high degree of variability in the results.

Growth rates in the controls and in the conditioned media were similar in almost all media conditioned at 0℃, although a higher growth rate was observed in Experiment 3 with *Kornmannia*. On the other hand, the relative growth rates varied with species in the media conditioned at 14℃. The same growth rate as a control occurred in the media conditioned by *Ulva*, *Colpomenia*, *Analipus* (in 2 of 3 experiments), *Palmaria* and *Phyllospadix*. A lower growth rate than a control occurred in the media conditiond by *Kornmannia* (in 3 of 4 experiments), *Monostroma*, *Alaria*, *Laminaria*, *S. thunbergii*, *Scytosiphon*, *Neodilsea* and *Neorhodomela* (in 2 of 3 experiments). The growth of the diatom was strongly inhibited in the media conditioned by *Laminaria* and Experiment 2 with *Alaria*.

Discussion

CRAIGIE and MCLACHLAN (1964) reported that yellow extracellular phenolic substances produced by *Fucus vesiculosus* showed a strong absorption with plateau with in the 260-280 nm and that all Phaeophyceae examined also produced yellow ultraviolet absorbing substances. These substances were toxic to unicellular algae (MCLACHLAN and CRAIGIE 1964).

Four of six phaeophycean algae (except for *S. confusum*) examined produced water soluble yellow ultraviolet-absorbing substances (Table 16) which were inhibitory to the growth of *B. obtusa* at 14℃. These results also indicate that the degree of diatom-inhibition by yellow ultraviolet-absorbing substances varied among these species.

It is known that some simple phenols isolated from green and red algae were highly toxic to unicellular algae (MCLACHLAN and CRAIGIE 1966) and the halogen-containing ketones from the red alga *Asparagopsis taxiformis* (DELILE) TREV. were extremely bacteria-and fungi-toxic (FENICAL 1974, 1975). Though some green and red algae scarcely released yellow ultraviolet absorbing substances (Table 16), the growth of *B. obtusa* was reduced in the media conditioned with 4 of 6 of these algae at 14℃. They may release some other water soluble substances which inhibit the growth of *B. obtusa*.

Sargassum confusum is one of the host algae for *B. obtusa* (Chapter II). In some experiments the growth of *B. obtusa* was suppressed in the medium conditioned by *S.*

Table 16 Optical density per cm of the conditioned seawater at 275 nm. Filtered seawater with tris (hydroxymethyl) aminomethane (1 g/liter) was used as a reference sample.

Species used to condition the growth medium	Exp. No.	Incubating condition of macroalgae 0℃, dark, pH 8	14℃, 5,000 lx, pH 9
Chlorophyceae			
Kornmannia zostericola	1	0.01	0.07
	2	0.01	0.06
	3	0.01	0.04
	4	0.02	0.12
Monostroma angicava	1	——[1)]	0.02
	2	——	0.02
Ulva pertusa	1	0.02	0.03
	2	0.02	0.04
	3	0.01	0.01
Phaeophyceae			
Alaria crassifolia	1	——	0.80
	2	——	1.88
	3	——	0.24
Analipus japonicus	1	——	0.28
	2	——	0.35
	3	——	0.40
Colpomenia bullosa	1	0.04	0.20
	2	0.02	0.14
	3	0.07	0.36
Laminaria japonica	1	——	0.42
	2	——	0.35
Sargassum confusum	1	0.13	0.19
	2	0.05	0.14
	3	0.07	——
	4	0.10	0.12
	5	0.05	0.13
	6	0.14	0.19
	7	0.04	0.12
	8	0.03	0.17
S. thunbergii	1	0.01	0.04
	2	0	0.02
	3	0.03	0.16
Scytosiphon lomentaria	1	——	0.98
	2	——	1.14
Rhodophyceae			
Neodilsea yendoana	1	——	0.02
	2	——	0.01
Neorhodomela aculeata	1	0.01	0.01
	2	0.01	0.01
	3	0.01	0.01
Palmaria palmata	1	0.02	0.02
	2	0.02	0.03
Angiosperm			
Phyllospadix iwatensis	1	0.03	0.05
	2	0.01	0.03
	3	0.03	0.04

[1)] Not examined.

confusum under the condition at the emersion of winter (*i. e.* 0℃ and darkness). Release rates vary with physiological changes in the plants themselves and seasonally. During a period of good growth 2.8-3 times as much material is excreted than during a period of poor growth (KHAILOV and BURLAKOVA 1969).

The experiments with *S. confusum* were carried out from February to May when this alga started to grow or was growing at Charatsunai (Chapter II). Uptake of excreted material during the growing stage might result in the suppression of the growth of *B. obtusa* in the media conditioned under the conditions to simulate winter (*i. e.* 0℃ and darkness). This explantion is problematic, however, because growth inhibition did not occur in both media at 0℃ and 14℃ in experiment-1, −5 and −8 when *S. confusum* was actively growing. Experiments 5 and 6 employed two different plants and were carried out on the same day and yielded conflicting results. There might be individual differences in the rate of release of inhibiting extracellular substances in *S. confusum*.

When the macroalgae which occur abundantly in both winter and spring were incubated under the winter emersion conditions they did not produce the extracellular substances which suppressed the growth of *B. obtusa*. However, when these macroalgae were incubated under the spring emersion conditions some of them produced the growth suppressing substances. Seawater temperature and pH are higher during emersion in spring than during the winter emersion (MIZUNO 1984a). Release rate may vary with external conditions according to KHAILOV and BURLAKOVA (1969). The production of yellow ultraviolet-absorbing substances increased linearly with increased pH (from pH 7 to pH 9) in *Fucus vesiculosus* (CRAIGIE and MCLACHLAN 1964). In some macroalgae which occur abundantly in both winter and spring, the environmental conditions during emersion of spring might cause the production of extracellular inhibitory substances. Most macroalgae occurring abundantly in spring released diatom-suppressing substances. The present study has demonstratd that *B. obtusa* is more frequently exposed to inhibitory substances at the emersion of spring than at the emersion of winter at Charatsunai shore.

Weekly observaions over 7 year period by PRATT (1966) showed that the phytoplankton of Narragansett Bay was alternately dominated, from May through October, by brief blooms of the diatom *Skeletonema costatum* (GREV.) CL. and the flagellate *Olisthodiscus luteus* CARTER. These taxa were almost never abundant simultanously. Then he carried out culture experiments and found that the diatom growth was inhibited by high concentrations of the flagellate-conditioned media, but was stimulated by low concentrations. From these results he showed that the mechanisms demonstrated in culture are operative in Narragansett Bay and influence the temporal succession of phytoplanktons. Furthermore, UCHIDA (1977, 1981) showed that the flagellate *Prorocentrum micans* EHR. released a substance which inhibited the growth of planktonic diatoms and proposed that the inhibitory substance may play a role in the seasonal succession of phytoplanktons.

Monthly observation over 4 years period shows *B. obtusa* occurs abundantly in

January-March and abruptly decreases in March-April (Chapter II). From the results of the field and culture studies, the extracellular products of macroalgae seem to be one of the factors governing the ecological behavior of *B. obtusa* at Charatsunai.

IV General Discussion

Autecological studies on the tube-dwelling diatom *Berkeleya obtusa* at Charatsunai, Muroran have been carried out in the field and the laboratory. Field observations (Chapter II) have clarified the the present diatom occur abundantly from November-December to March-April and sporadically from July to September in the lower littoral zone (below + 60 cm of the tidal datum line). The influences of several selected environmental factors (physical, chemical and bioligical) on the growth or survival of this diatom have been investigated in laboratory experiments and the relationship between the influence of each environmental factor and the ecological behavior of this diatom has been discussed (Chapter III).

In late autumn and winter (Nov.-Feb.), the seawater temperature during submersion is within the opimum range for growth (Nov.) or the lower (Dec.-Feb.) (Chapter III-1). The nutrients in the seawater are sufficient for the growth (Chapter III-2). The emersion of the lower littoral zone occurs in the nighttime during these seasons. Since the temperature of air and the evaporation rate of water are low at nighttime in these seasons, desiccation during emersion may scarcely damage the cells of the diatom (Chapter III-5). Air temperature drops below the freezing point during emersion, but it is not so cold that this diatom cannot survive (Chapter III-5). Since the lower littoral zone is nearly submerged in the daytime (MIZUNO 1984a), the diatom is not exposed to strong desiccation. The pH and salinity of the water in tide pools in the lower littoral zone are mostly within the optimum range for the growth (MIZUNO 1983, Chapter III-3 and -4). Water soluble extracellular products of macroalgae during emersion during these seasons have influenced scarcely on the growth of the diatom (Chapter III-6). Thus the laboratory experiments have shown that the environments of the lower littoral zone during these seasons are adequate to allow the occurrence of *B. obtusa*.

On the other hand, in the upper and middle littoral zones in late autumn and winter, prolonged exposure to the atmosphere may damage the cells of the diatom. The duration of emersion in these zones exceeds the tolerance limit to desiccation, even under the low desiccating conditions in these seasons (Chapter III-5). High salinity suppressing the growth is sometimes observed in the tide pools in these zones (MIZUNO 1984a, Chapter III-4). Laboratory experiments have shown that the occurrence of the diatom is prevented by the conditions of the upper and middle littoral zones during these seasons.

In spring (Mar.-May), the seawater temperature during submersion is the lower or near the optimum range for growth (Chapter III-1). However, the nutrients in the seawater are insufficient for the growth (Chapter III-2). The lower low water of spring tide occurs in the

daytime from spring to early autumn. As a result, the shore during emersion is characterized by a high desiccation. Temperature and pH of water in tide pools become high (MIZUNO 1984a). Except in the lower part of the growth zone these factors may cause damage to the cells of the diatom (Chapter III-1, −3 and −5). Extracellular products of macroalgae during emersion have a tendency to suppress the growth of the diatom (Chapter III-6). In summer (June-Aug.), the seawater temperature during submersion is near or exceeds the tolerance limit for the growth (Chapter III-1) and the poor nutrient conditions continue in summer (Chapter III-2). Furthermore, high desiccation rates of atmosphere or high temperatures and the high pH of water in the tide pools during emersion may damage the cells (Chapter III-1, −3 and −5). In early and middle autumn (Sep.-Oct.), the low nutrients continue to exist (Chapter III-2). The high seawater temperature during submersion continues in September (Chapter III-1). Although the lower low water of spring tide occurs in the nighttime in October, the temperature of air and the evaporation rate are still high in the nighttime. Except for cells in the lower part of the growth zone this desiccation may damage the cells (Chapter III-5). There is a probability that a low salinity caused by rain at emersion adversely affects the cells during warm season (Chapter III-4). Laboratory experiments have shown that the diatom does not occur under the conditions characterizing the shore environment from spring to middle autumn. The results of the laboratory experiments suggest that the environmental factors examined govern the seasonal occurrence and the vertical distribution of the diatom at Charatsunai, although the sporadic occurrences at the shore from July to September is not explained by the results of laboratory experiments.

The colonies of this diatom appear sporadically at the shore during warm months. It is difficult to believe that individual cells have grown into large colonies at the shore during this period, since the shore environment is thought to be unfavorable for the growth at this time. ALEEM (1950) suggests that persistence in the sublittoral zone is one of the modes of survival of littoral diatom during the period of their absence from the shore. In Uchiura Bay (Volcano Bay), a seawater temperature of nealy 10℃ was recorded in the zone below 15 m depth during the warm months (OHTANI *et al.* 1971a, 1971b, NISHIHAMA *et al.* 1979). This temperature is within the optimum range for the growth of the diatom (Chapter III-1) and once a large number of colonies were observed in the sublittoral zone (Chapter II). The laboratory experiments suggest that some of the physiological properties of the diatom are similar to those of sublittoral macroalgae (Chapter III). The light intensity of saturation for the growth rate is near to that of sublittoral macroalgae. The diatom can grow at a low light intensity of 170 lx. Thus it is possible that the diatom grows in the sublittoral zone during warm months.

Observation on twining of colonies *in situ* and in the laboratory have been shown that this diatom initially appears on spinous substrates in a large colony which is easily visible with the naked eye. The colonies observed at the shore during warm months are regarded as drifting fragments of colonies which have grown up in the sublittoral zone and which have

been transported shoreward.

I cannot confirm that the present diatom grows in the sublittoral zone during warm months, although some indirect evidence has been obtained in both field and laboratory experiments. If it is true, however, how do the colonies in the sublittoral zone appear at the shore? The Tugaru Warm Current reaches the northeastern mouth (Muroran) of Uchiura Bay in June-July and begins to enter through the mid-and lower-water layers from August September (OHTANI *et al.* 1971b). Perhaps the current separates the colonies from their substrate and carries up to the shore. Clearly further studies are necessary to elucidate the ecological behavior of the diatom in the sublittoral zone and the mechanism responsible for their appearance at the shore.

All strains of the diatom isolated have failed to produce auxospore in laboratory cultures. Auxospores were observed once in the field collection of October. Cells cultured under the condition of the same temperature and daylength as October failed to induce auxospore formation. The problem of the induction of auxospore formation in the laboratory remains unsolved.

Summary

Autecological studies on the marine tube-dwelling diatom *Berkeleya obtusa* at Charatsunai, Muroran have been carried out in the field and in the laboratory.

1. *B. obtusa* occurred mostly on *Neorhodomela aculeata*, *Sargassum confusum* and *S. thunbergii* in the lower littoral zone. *B. obtusa* initially appeared on the substrates as a large colony. Colonization occurred most often on substrates with spine-like structures which permitted twining. The highest densities were observed from November-December to March-April. The annual maximum standing crop was observed from January to March. Sporadic occurrences of low abundance were observed from July to September. The production experiment showed that *B. obtusa* colonized the substrate from late October to early March. Auxospores were observed once in the material collected in October 1978. The valve length of gametangia ranged from 18.3 to 20.9 μm ($\bar{X}\pm SD=19.8\pm0.8\,\mu m$; n=30) and the length of initial cells ranged from 31.6 to 38.5 μm ($\bar{X}\pm SD=35.6\pm1.9\,\mu m$; n=30).

2. The growth rate and survival of *B. obtusa* were examined under various combinations of temperature, daylength and light intensity. The optimum temperature for growth was 10-14℃. *B. obtusa* grew slowly at 5℃, very slowly at 18℃ and did not grow at 22℃. Experimental evidence suggests that the growth rate may be saturated at a low light intensity of near 1,000 lx. Differences between growth rates in long and short daylengths were barely evident except at 18℃. *B. obtusa* can grow at a very low light intensity of 170 lx. *B. obtusa* survived for 4 months in complete darkness, and for 6 months at 170 lx and 3,000 lx. Four epiphytic diatoms growing on a colonial tube of *B. obtusa* grew faster than *B.*

obtusa. The monthly mean seawater temperatures during the period of the main occurrence (Nov.-Apr.) were lower than the optimum for growth and exceeded the tolerance limit for growth during its sporadic occurrences (July-Sep.). On the other hand, the mean seawater temperatures when the diatom was not observed (May-June and Oct.) were near or within the optimum for growth.

3. The influence of nutrients in seawater on the growth was examined using the method of nutrient-enrichment. The potential of seawater in Charatsunai for growth of *B. obtusa* varied seasonally. The potential was high in December and January and low in other months. The period when the high potential of seawater for growth was obtained approximately coincided with the main occurrence period of the diatom at study site. The growth rate in the unenriched seawater (U) was lower than that in the seawater enriched with all nutrients (ALL) in all months. It was necessary for obtaining the same growth rate as ALL to supply with Fe and/or vitamin B_{12}, even though the December and January waters had high potential for growth. The same growth rate as ALL was obtained by adding phosphate to July, September and March seawaters, phosphate (or silicate?) to February seawater and silicate with nitrate or with phosphate to April seawater. Superaddition of Fe and/or vitamin B_{12} was necessary in July and February seawaters.

4. The influence of daily short-term exposure to high pH (8.9-9.0) on growth was examined in laboratory. A daily exposure for 6 hours or more suppressed the growth, even though exposure for 3 hours scarecely suppressed growth. These results suggest that high pH might affect growth from March to July at the level of +40 cm of the tidal datum line at the shore, from March to September at +60-+80 cm and throughout the year at +160 cm, while high pH may not affect growth throughout the year at +10 cm.

5. The influence of daily short-term exposures to high (39-41‰ of S) or low (14‰) salinity on growth was examined in laboratory. A daily exposure to high salinity for only one hour suppressed growth. Growth failed in all cultures exposed to low salinity. These results suggest that low salinity at emersion during warm season may suppress the occurrence of the diatom and high salinity at emersion during cold season may suppress the occurrence at the high tidal level of the shore.

6. The survival of *B. obtusa* under various combinations of temperature and evaporation rates were examined in the laboratory. Under the condition of no evaporation, the diatom survived for at least 7 hours in the range from −13℃ to 22℃, At −13℃ the diatom survived for 5 hours with an evaporation rate of 0.16 g/28 cm²/hr. At 5℃, it survived for 3 hours at 0.24 g/28 cm²/hr and for 2 hours at 0.32 g/28 cm²/hr. At 14-22℃, it died within 3 hours at 0.10 or 0.14 g/28 cm²/hr and within 1 hour at 0.52 or 0.66 g/28 cm²/hr. These results suggest that *B. obtusa* cannot survive during emersion in June at the level of +10 cm, during emersion from March to October at +40 cm, and during emersion throughout the year at +60 cm or above.

7. The influence of extracellular substances on the diatom growth produced by various

macroalgae under conditions of emersions during winter and spring was examined in the laboratory. When the macroalgae which occurred abundantly in both winter and spring were incubated under winter emersion conditions, they rarely produced extracellular substances which suppressed the growth of *B. obtusa*. However, when they were incubated under spring emersion conditions, some of macroalgae produced growth suppressing substances. Most macroalgae which occurred abundantly in spring released growth suppressing substances under the conditions of spring emersion.

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PLATE I

Attachment of *Berkeleya obtusa* to various substrates.

A, B. To *Neorhodomela aculeata.*

C, D. To *Sargassum confusum.*

E, F. To *Sargassum thunbergii.*

Scale in A for all=1 mm.

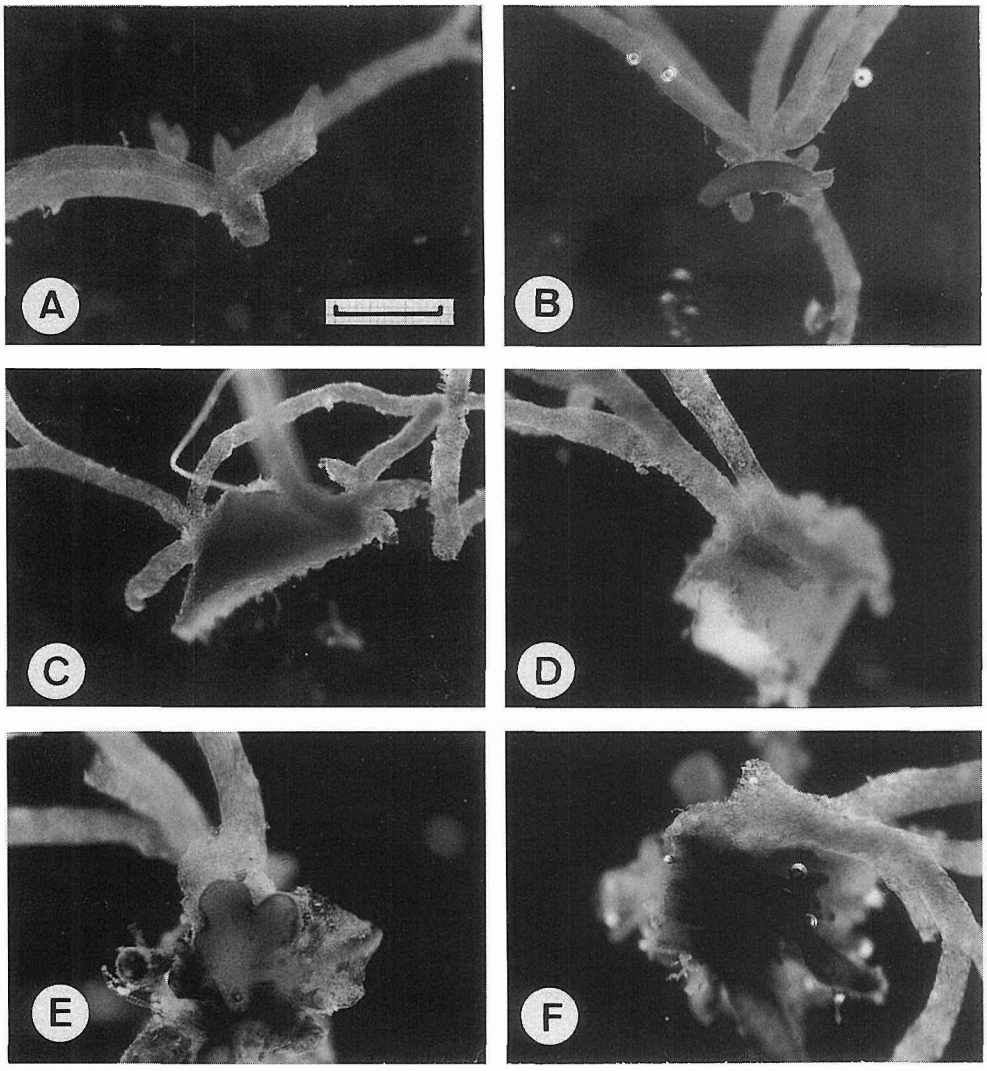

A
B
C
D
E
F

PLATE II

Colonies of *Berkeleya obtusa* on macroalgae in a short term *in situ* experiments.

A. Coiled colonies on *Sargassum confusum*—17-19 December, 1979.
B. Detail of A.
C. Coiled colonies on *S. confusum*—29-30 December, 1979.
D. Detail of C.
E. Coiled colonies on *Sargassum thunbergii*—2-4 January, 1980.
F. Detail of E.
G. A coiled colony on *S. confusum*—17-19 December, 1979.
H. A coiled colony on *Neorhodomela aculeata*—2-4 January, 1980. Scales in A, C and E= 5 mm; scales in B, D, F and G=3 mm; scale in H=2 mm.

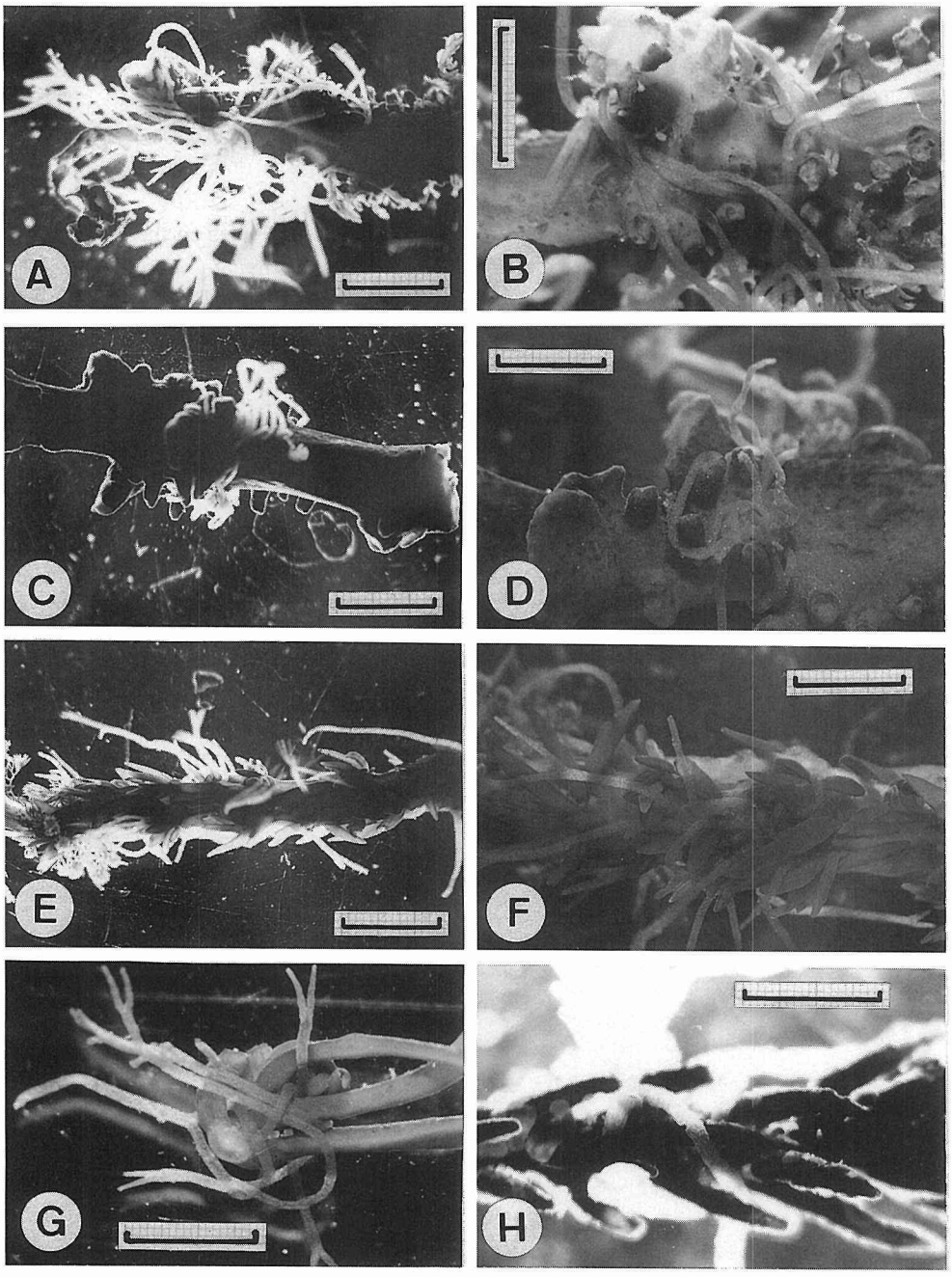

A
B
C
D
E
F
G
H

PLATE III

Twining of colonies of *Berkeleya obtusa* observed in the laboratory.

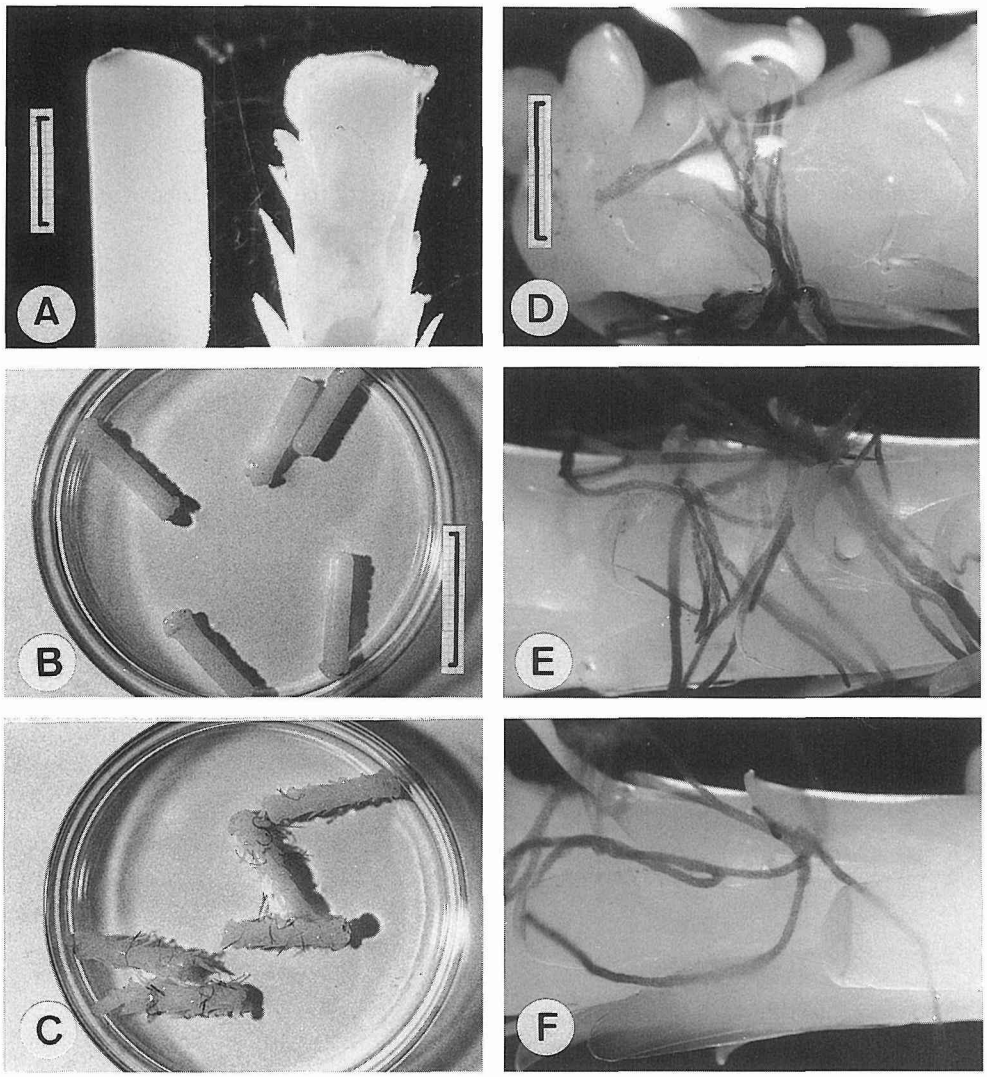
A. The two kinds of surface of the polyethylene rods, incised surface (right), smooth surface (left).

B. Rods with a smooth surface after 5 min-stirring.

C. Rods with incised surface after 5 min-stirring. Note coiled colonies on the rod.

D—F. Coiled colonies on rods with incised surface after 5 min-stirring.

Scale in A for A=5 mm; scale in B for B, C=3 cm; scale in D for D—F=3 mm.


A
D
B
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C
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PLATE IV

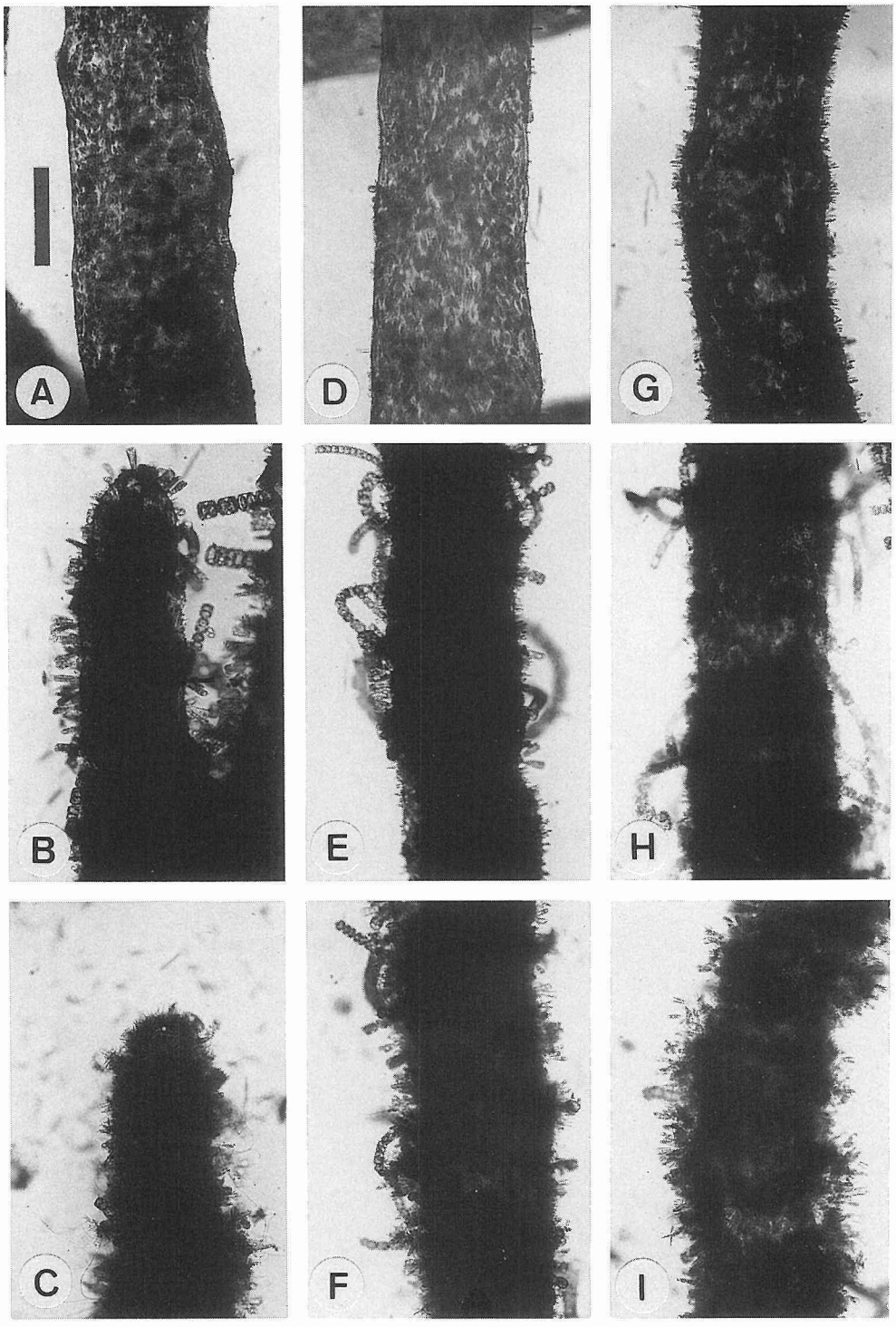
Seasonal changes in epiphytic diatoms on the surface of the colonial tube of *Berkeleya obtusa*.

A—C. Materials growing on *Neorhodomela aculeata*.

D—F. On *Sargassum confusum*.

G—I. On *Sargassum thunbergii*.

A, D, G: collected in January 1977; B, E, H: in February 1977; C, F. I: in March 1977. Scale in A for all=200 μm.


A
D
G
B
E
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C
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