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Comparative Morphology and Seasonality of *Campylaephora borealis* and *C. crassa* (Ceramiaceae, Rhodophyta)

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Populations of *Campylaephora borealis* (Nakamura) Seo, Cho et Boo and *C. crassa* (Okamura) Nakamura show a year-around occurrence of all life-history stages. Such a concurrency of life-history stages produces problems in recognizing species in the field. Here, we invesitgated the morphological variation and life-history stages of both species using a statistical character analysis. Life-history stage was correlated with the seawater temperature in *C. borealis*, whereas it was dependant on biomass in *C. crassa*. Thalli had dichotomous branches with adaxial branchlets. The statistics showed that the seasonal change in morphology of *C. borealis* was significantly different from that of *C. crassa* in seven qualitative characters and five quantitative characters ($p < 0.001$), although six quantitative features including tetrasporangial size were similar in both species. The morphological difference between the two species may be due to the annual variation of branchlet number and the variance of branch subangle.

Key Words: *Campylaephora borealis*, *C. crassa*, morphology, Rhodophyta, seasonality, statistical character analysis

INTRODUCTION

Genus *Campylaephora* was established on the basis of specimens named as *Ceramium rubrum* var. *firmum* by J. Agardh (1851). The genus includes four species; *C. borealis* (Nakamura) Seo, Cho et Boo, *C. crassa* (Okamura) Nakamura, *C. hypnaeoides* (Okamura) Nakamura, and *C. japonica* Noda (Nakamura 1950, 1965; Boo and Yoshida 1991; Seo *et al*. 2003). *Campylaephora borealis* was classified as one of the five forms in *C. crassa* (Nakamura 1950; Itono 1977), but it was recently separated from *C. crassa* based on the basiphyte range and the plastid DNA data (Seo *et al*. 2003). Although *C. borealis* is very similar to *C. crassa* and both species occur on the east coast of Korea (Boo and Lee 1994; Seo *et al*. 2003), *C. borealis* is more closely related to *C. hypnaeoides* than *C. crassa* (Seo *et al*. 2003).

The sporophyte and the gametophyte in *Campylaephora borealis* and *C. crassa* occurred in a same season (Boo *et al*. 1991; Boo 1992), although *C. hypnaeoides* has a clear seasonality (Okamura 1922, 1927; Notoya 1979). Such a co-ocurrence of gametophytes and sporophytes may be advantageous in harsh ocean conditions. Even in isomorphic species the slight difference in the adult phase or among juveniles may play an important ecological role (Hawkes 1990; Houghes and Otto 1999) and help maintain the population from genetic damage (Long and Michod 1995). For example, haploids are efficient replicators, while diploids are resistant to damage, wherein the sexual stage may combine the advantage of both (Engel *et al*. 2004). This study was undertaken to compare detailed morphological variation of *C. borealis* and *C. crassa*, and to trace the seasonal changes of the life-history stages in the field using statistical character analysis.

MATERIALS AND METHODS

Sampling and seasonality

Plants were sampled bimonthly in Sinnam (N37°20′, E129°3′) and in Guryongpo (N35°70′, E129°4′) on the eastern coast of Korea by the method described in Scheaffer *et al*. (1986). On the rocky side of the Sinnam site, *Campylaephora borealis* occurred parallely with sea-water level. Because *C. crassa* rarely occurred in Sinnam, we chose Guryongpo site for *C. crassa* (Boo *et al*. 1991). Surface water temperature was measured three times per

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Table 1. Peason product-moment coefficient of correlation between ecological and biological data of *Campylaephora borealis* and *C. crassa*.

	C. borealis				*C. crassa*			
	RF	Temp.	D/W	Density	RF	Temp	D/W	Density
RF	1				1			
Temp.	0.777	1			-0.653	1		
D/W	0.707	0.781	1		0.428	-0.100	1	
Density	0.643	0.735	0.346	1	0.224	0.382	0.638	1

D/W, dry weight (g); RF, reproductive frequency (%); Temp., seawater temperature (°C)

every collection and a median value was taken.

To compare the seasonality, thalli having reproductive organs were counted, but thalli under 2 cm in length were excluded. The total number and the wet weight of thalli in each 10 × 10 cm^2 size quadrat was used as an estimation of density and biomass, respectively. We calculated Pearson's correlation coefficients between morphological and ecological data taken in the present study.

Statistical analysis of morphological characters

A total of 366 thalli were collected in the field. We sorted out thalli having reproductive organs and measured the wet weight of each thallus. Ninteen features (Fig. 1; Tables 2 and 3) were analyzed with the Statistical Analysis System (SAS Institute 1985; Release 6.03). For qualitative characters, the value of 0 or 1 was given. To test the difference of two species, a Chi-Square test was done at the 10% significance level (Kleinbaum *et al.* 1988). T-test was applied for eleven quantitative characters. One-way analysis of variance (one-way ANOVA) and two-way analysis of variance (two-way ANOVA) were used between two species and between sampling months (Bowerman and O'Counell 1990). Standardized principal components analysis (PCA) based on a correlation matrix was done to compare the seasonal data of the two species (Kleinbaum *et al.* 1988). Five quantitative characters showing the significant difference between two species were used for the PCA. Operational taxonomic units (OTU's) were 12 sampling months.

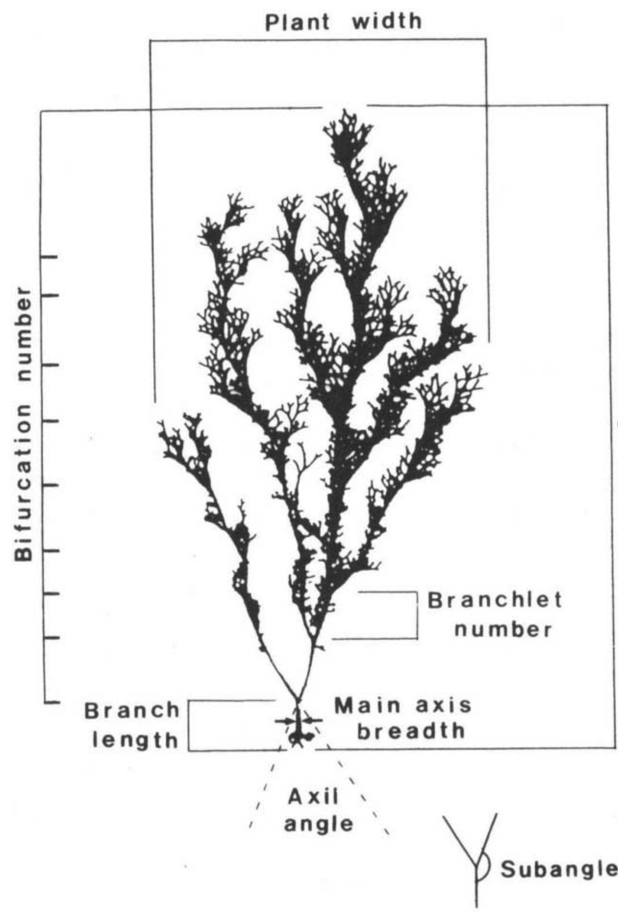


Fig. 1. Morphometric characters used in the present study.

RESULTS

Seasonality

Surface sea-water temperature was in a range of 7-23°C in Sinnam and 10-24°C in Guryongpo (Fig. 2A).

The density (the number of thalli per each quadrat) was dependent on sampling month (Figs 2B, C). For *Campylaephora borealis*, the maximum dentity was 56 thalli in August and the minimum was seven thalli in April (Fig. 2B). The wet weight reached the maximum in June with 91.6 g per quadrat and dropped to 29.6 g in October (Fig. 2B). The gametophyte predominated from December to April. Many thalli having tetrasporangia occurred from February to October. The gametophytes predominated again in August (2D).

For *C. crassa*, the maximum number of thalli was in August with an average of 101 thalli per quadrat, but

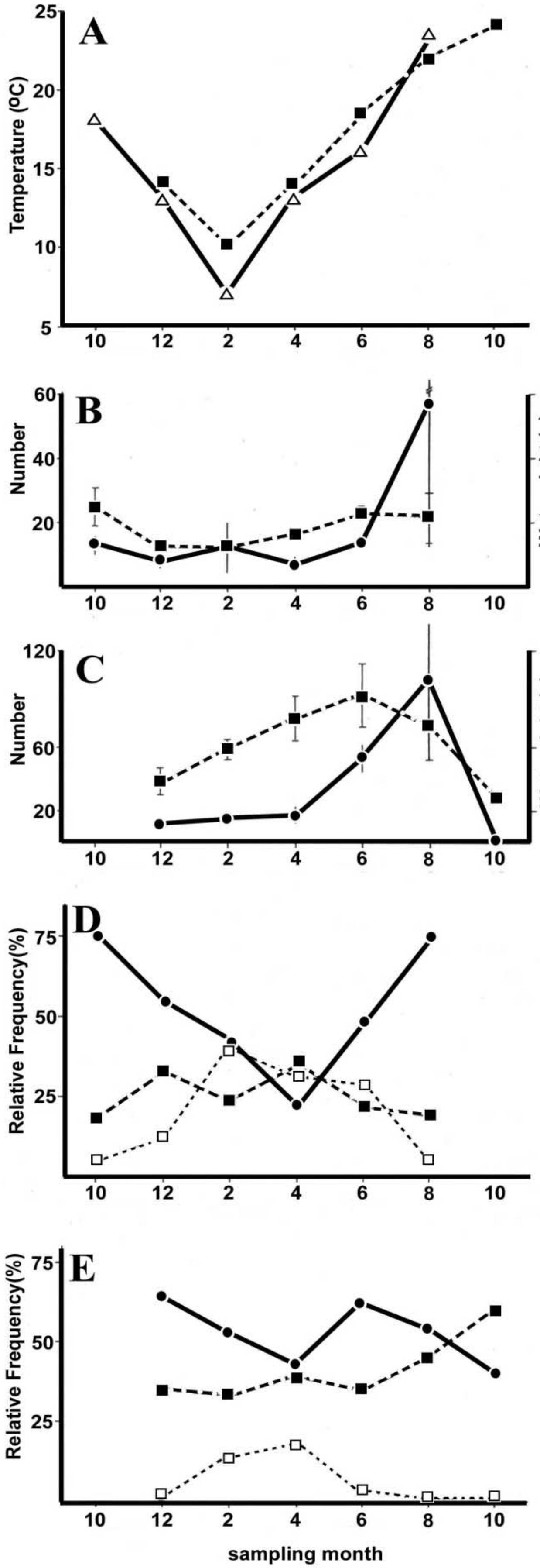
Table 2. Chi-Square test for qualitative characters of *Campylaephora borealis* and *C. crassa*. Parenthesis indicates percentages of specimens

Characters	*C. borealis*	*C. crassa*	DF	Value	P-value
Plant habit (n = 336)					
spread	13 (7)	148 (95)	1	257.3	0.000
narrow	167 (93)	8 (5)			
Adherence on paper (n = 336)					
no	33 (18)	10 (6)	1	10.6	0.001
yes	147 (82)	146 (94)			
Alternate branchlet (n = 336)					
no	1 (1)	133 (85)	1	250.1	0.000
yes	179 (99)	23 (15)			
Appearance (n = 336)					
single	156 (87)	115 (74)	1	9.0	0.003
Combined	24 (13)	41 (26)			
Proliferous branchlets (n = 336)					
adaxial side	73 (41)	74 (47)	1	1.6	0.205
all side	107 (59)	82 (53)			
Touch (n = 336)					
tender	159 (88)	113 (72)	1	13.7	0.000
harsh	21 (12)	43 (28)			
Tetrasporangia (n = 83)					
whorled	29 (97)	1 (2)	1	77.4	0.000
scattered	1 (3)	55 (98)			
Epiphyte (n = 336)					
absent	40 (22)	152 (97)	1	193.0	0.000
present		140 (78)	4 (3)		

Table 3. Simple statistics for quantitative characters in *Campylaephora borealis* and *C. crassa*

characters	*C. borealis*	*C. crassa*	F-test	distribution	P-value	result
Plant length	4.6 ± 2.6	3.6 ± 1.7	0.0000	unequal	0.0001	different
	(n = 589)	(n = 957)				
Plant width	3.6 ± 2.4	3.5 ± 1.9	0.0000	unequal	0.7110	similar
	(n = 589)	(n = 957)				
Axis breadth*	0.5 ± 0.3	0.6 ± 0.3	0.9288	equal	0.0321	similar
	(n = 180)	(n = 156)				
Branch length*	1.3 ± 1.7	1.0 ± 1.0	0.0000	unequal	0.0381	similar
	(n = 180)	(n = 156)				
Bifurcation*	7.8 ± 4.4	5.3 ± 2.7	0.0000	unequal	0.0001	different
	(n = 180)	(n = 156)				
Axil angle*	53.9 ± 18.3	85.9 ± 21.4	0.0438	equal	0.0001	different
	(n = 180)	(n = 156)				
Subangle*	142.3 ± 12.1	127.4 ± 13.3	0.2216	equal	0.0001	different
	(n = 180)	(n = 156)				
Branchlet*	7.7 ± 14.2	16.9 ± 12.2	0.0646	equal	0.0001	different
	(n = 180)	(n = 156)				
Tetrasporangia	1.2 ± 0.2	1.2 ± 0.1	0.0032	unequal	0.0445	similar
	(n = 102)	(n = 284)				
Carpospore	1.8 ± 0.5	1.7 ± 0.5	0.2363	equal	0.0854	similar
	(n = 83)	(n = 145)				
Cortical cell	1.5 ± 0.4	1.5 ± 0.4	0.4548	equal	0.3064	similar
	(n = 154)	(n = 590)				

*Data from herbarium specimens



only single thallus occurred in October (Fig. 2C). The maximum wet weight was 25.0 g in October and the minimum value was 11.8 g in February (Fig. 2C). Sterile vegetative thalli were less than 5% of total thalli (Fig. 2E).

Campyalephora borealis often grew on *Prionitis divaricata* and other related red algae. Many epiphytes such as *Porphyra* and *Ulva* species were found on thallis of *C. borealis*. However, *C. crassa* always grew on a brown alga, *Sargassium yezoense*, and a couple of epiphytes such as *Ulva* and *Polysiphonia* species were found on the thallus of *C. crassa* (Fig. 3).

The correlation coefficient between temperature and seasonality data such as the number and wet weight of thalli are shown in Table 1. The life-history stage of *C. borealis* was correlated with surface sea-water temperature, wet weight, and density of thalli ($r > 0.64$, respectively). However, life-history stage of *C. crassa* showed a negative correlation with surface sea-water temperature ($r = -0.65$) and a positive correlation with wet weight ($r > 0.60$).

Morphological variation

The thallus length of *C. borealis* averaged 4.6 ± 2.6 cm (n=577) (Fig. 4). The sporophytes were 10 ± 5 cm in April, with a maximum of 22 cm. In the same month, the maximum of female gametophytes was 16 cm. Both male gametophytes and vegetative thalli showed a maximum length in October, respectively. Axil was acute with angles of 26 to 72 degrees and subangle was 130 to 154 degrees. Bifurcation number was 3 to 18 times, and branchlet number in the third branch was 0 to 22 (Table 3). From ANOVA tests, thallus length, thallus width, bifurcation number, axil angle, and branchlet number were significantly different, depending on sampling months ($p = 0.0001$). Main axis breadth and branch length did not show any seasonal difference (Table 4, $p > 0.02$).

The avarage length of *C. crassa* was 3.6 ± 1.7 cm (n =

Fig. 2. Bimonthly changes of ecological and biological elements of *Campylaephora borealis* and *C. crassa*. A. Surface seawater temperature in Sinnam (solid line) and Guryonpo (dotted line). B. Number of thalli (solid line) and wet weight (dotted line) of *C. borealis*. C. Number of thalli (solid line) and wet weight (dotted line) of *C. crassa*. D. Relative frequency of sexual (solid line), tetrasporangial (dotted line with closed squares), and vegetative (dotted line with open squares) thall of *C. borealis*. E. Relative frequency of sexual (solid line), tetrasporangial (dotted line with closed squares), and vegetative (dotted line with open squares) thall of *C. crassa*. Error bars indicate standard deviations.

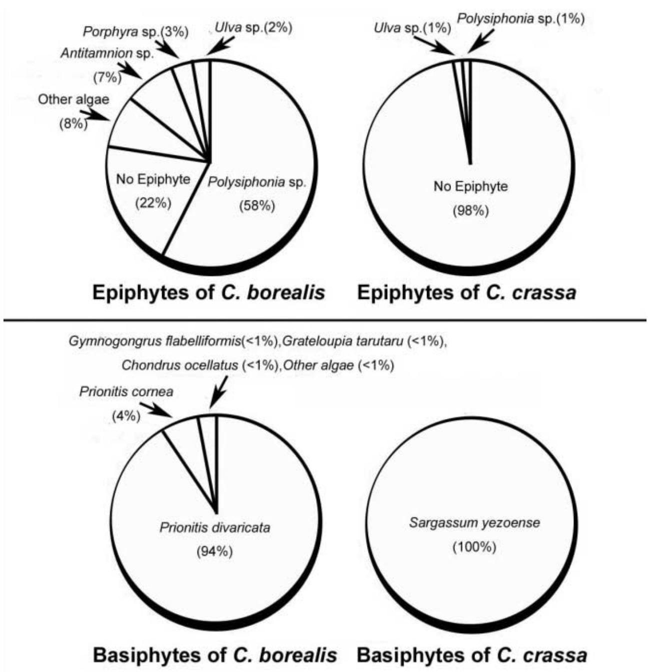


Fig. 3. Epiphytes and basiphytes (%) of *Campylaephora borealis* and *C. crassa*.

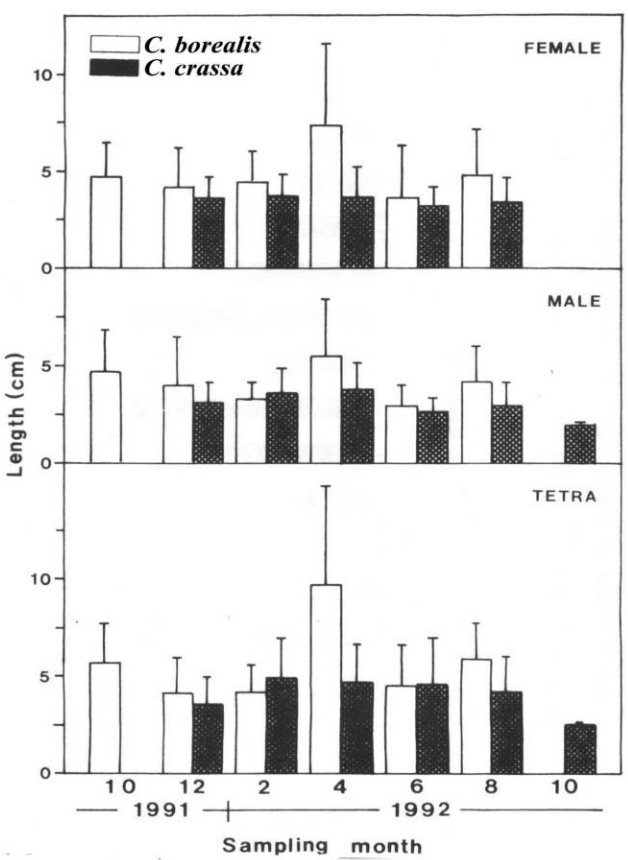


Fig. 4. Bimonthly changes of plant length of *Campylaephora borealis* (white bar) and *C. crassa* (black bar). Error bar indicates standard deviations.

Table 4. F statistics in the analysis of variance for the three partitioned tests between sampling months

characters	sampling month	*C. borealis*	*C. crassa*
Plant length	0.0001	0.0001	0.0001
Plant width	0.0001	0.0001	0.0940
Main axis breadth	0.0313	0.5641	0.0395
Branch length	0.0093	0.0287	0.3330
Bifurcation number	0.0001	0.0001	0.0218
Axil angle	0.0001	0.0001	0.2599
Subangle	0.0001	0.0213	0.7778
Branchlet number	0.0001	0.0001	0.0653

Table 5. Characters, sample sizes, and loadings of Principal Components Analysis

characters	sample size	component 1	component 2
Subangle	336	0.462435	-0.445027
Bifurcation number	336	0.462128	0.465334
Plant length	336	0.395261	0.564841
Axil angle	336	-0.440400	0.515990
Branchlet number	336	-0.471602	0.011164

922). There were not found differences among the sampling months and life-history stages. The maximum length of tetrsporophytes was 13 cm and female gametophytes 9 cm in April. The maximum length of male gametophytes was 7 cm in February. Axil angle, bifurcation number, and branchlet number of *C. crassa* was higher than those of *C. borealis*. The ANOVA tests (Table 4) show that most characters of *C. crassa* are similar over seasons ($p > 0.02$). *Campylaephora crassa* was similar to *C. borealis* in six other quantitative characters ($p > 0.03$, Table 3).

In PCA, the principal component 1 had 64% of total variance and the principal component 2 had 26% of total variance (Table 5). Most information of the OTUs was reflected with two principal components (Fig. 5). The principal component 1 had the Eigen Value of above 4.0, depending on the bifurcation number and the subangle. The principal component 2 was conflicted with axil angle (Table 5). By the principle component 1, the OTUs of *C. borealis* were tilted on the right side, and those of *C. crassa* were positioned on the left side. With the respect of the principal component 2, the OTUs of *C. borealis* were dispersed widely because of the variance of plant length and axil angle. On the other hand, the OTUs of *C. crassa* were aggregated.

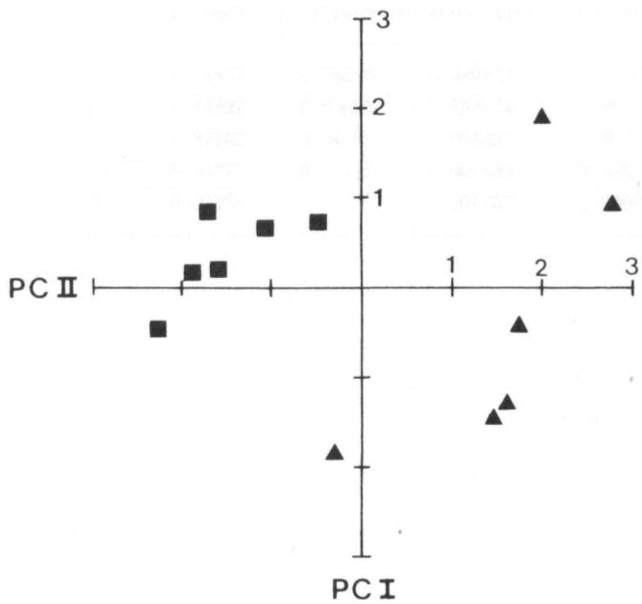


Fig. 5. The projection of OUTs onto the first two principal components. Triangles are representatives of *Campylaephora borealis* and squares are *C. crassa*.

DISCUSSION

Seasonality is annually recurring life events that are made up of the biological properties such as growth and reproduction and the periodical changes of the environmental factors (Lieth 1974). The study of seasonality can provide an important information of ecological populations (Edwards and Richardson 2004) and the level of response to climate change may vary across species. The decoupling of seasonality would be important ramifications of populations (Berry and Lembi 2000; Kitzes and Denny 2005).

The present study shows that *C. borealis* is clearly different from *C. crassa* in seasonality and density, although both occurred in the same locations (Boo *et al.* 1991). The gametophytes of *C. borealis* were abundant during winter and decreased in spring. On the other hand, the sporophytes of *C. crassa* were abundant in late summer (Fig. 2E). It is concluded that *C. borealis* starts to grow from fall and has a maximum growth during spring, while the maximum growth period of *C. crassa* is in early summer. The size of sexual structures such as the tetrasporangia and cystocarps shows no seasonality in both species.

Algal seasonality may be changed by biological and physical factors, which affect the efficiency of the photosynthesis (Kain and Norton 1990). The life-history stages of *C. borealis* were positively associated with temperature, while the reproduction of *C. crassa* was more related with low temperature than high temperature. The maximum length in the period of the low water temperature strongly suggests that phyical factor affecting thallus growth may be surface-water temperature. *C. borealis* is a cold-temperate species which spreads to the north, whereas *C. crassa* is a warm-temperate species, being distrubuted to the south (Boo 1985; Boo *et al.* 1991; Seo *et al.* 2003).

Basiphytism may be a contributor for the life-history stage of *Campylaephora* species. In *C. crassa* having a single basiphyte, life-history stage and density were more correlated with biomass (as wet weight) than surface water temperature. Two species showed different colors. *Campylaephora borealis* was pinky, in color, and *C. crassa* was yellowish. According to Gonzalez and Goff (1989), blade color of two *Microcladia* species, which are very similar to *Campylaephora*, might be physiologically effected by basiphytes. Therefore, the basiphyte may be an effector for the growth of *C. crassa*. The morphological variation of the intertidal red algae to the different forces of waves has been reported (Kitzes and Denny 2005) and the phenological differences of holdfast attachment have been reported in other red and brown algae (Waaland 1990; Milligan and DeWreede 2000).

Since it is correlated with the primary growth and angle of the apical cell (Moe and Silva 1979; Waaland 1990), bifurcation pattern of branches has been used as a stable diagnostic character for recognizing species. In the genus *Ceramium*, the bifurcation has been used for a numerical analysis of the growth (Bruno and Eklund 2003). Branchlet number, bifurcation number, and branch angle of *C. borealis* were more variable than those of *C. crassa*. In contrast to the previous morphological studies (Nakamura 1954), there was no significant difference on the position of branchlets between *C. crassa* and *C. borealis*.

The morphological differences in *C. borealis* and *C. crassa* are found in the number and interval of branchlets rather than the distribution of branchlets. The variance of branch subangle is also discontinuous. The present results suggest the existance of the discontinuous variation of morphology and life-history stages between the two species. The PCA clearly identifys two species not only by the qualitative character but also by the quantitative characters.

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