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Seasonal Fluctuation in a Marine Red Alga, *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta), from Nokonoshima Island, Southern Japan

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Seasonal fluctuation of *Gracilaria vermiculophylla* population was investigated at Nokonoshima Island, Fukuoka, southern Japan from November 2008 to April 2010. The wet weight and the length of randomly collected five plants were monthly measured, and the size class distribution of the excised thalli (categorized into nine size classes based on the thallus length) was followed. The proportions of reproductive types (spermatangial, cystocarpic or tetrasporangial) were also followed for one year (from May 2009 to April 2010) on additional collection to make a total of 100 plants. As the results, the mean wet weight and thallus length were highest in spring (April to June) and lowest in autumn (September to November). The size class distribution fluctuated greatly throughout the year and the smallest size class (Class I; <5.0cm) was conspicuous from October to December both in 2008 and 2009, while the largest size class (Class IX; 40.0–44.9 cm) was only found on May 2009, reaching 41.2 cm in length. The proportions of the three types of plants fluctuated throughout the year with the tetrasporangial plants constantly present and mostly dominant. From these results, it was known that new thalli started to grow from October, and that they could mature rapidly even in short size, suggesting that the renewal of the population is made yearly on sporic basis (tetraspores and carpospores).

Key words: *Gracilaria vermiculophylla* population, Rhodophyta, seasonal fluctuation, size class distribution, Nokonoshima Island

INTRODUCTION

The genus *Gracilaria* Greville comprises marine red algae, which play an important role in brackish and marine ecosystems. Some species are harvested commercially as a major source of agar and as a food for human and aquatic animals (Nelson, 1989; Bird, 1995). So far, more than 150 species of the genus have been described from all over the world (Guiry and Guiry, 2014), including 20 species in Japan (Yoshida and Yoshinaga, 2010).

Gracilaria vermiculophylla (Ohmi) Papenfuss was originally classified by Ohmi (1956), assigning in the genus *Gracilariopsis* Dawson on the basis of the specimens collected from Hokkaido, Japan, but subsequently was transferred to *Gracilaria* by Papenfuss (1967). Traditionally this species has been classified based on its small number of nutrient tubular filaments in the cystocarp, the arrangement of spermatangia in deep cavity, and the small cells with dense protoplasts in gonimoblast tissues (Terada and Yamamoto, 2001; Rueness, 2005). *G. vermiculophylla* is widely distributed in the Northern Hemisphere (Terada and Yamamoto, 2001; Bellorin *et al*., 2004; Rueness, 2005; Freshwater *et al*., 2006; Thomsen *et al*., 2007; Skriptsova and Choi, 2009). In the Northern Atlantic Ocean and along the European coast, as an invasive species that recently become a serious problem for commercial fishing and environmental impacts, the ecological role of this alga has been studied (Freshwater *et al*., 2006; Thomsen *et al*., 2007, 2009; Weinberger *et al*., 2008). In Japan, however, there have been few such studies (Terada *et al*., 2000; Phooprong, 2005) despite the fact that it is endemic to the northwestern Pacific (Terada and Yamamoto, 2001; Yang *et al*., 2008).

The present study examined the seasonal fluctuation and population trends of G. vermiculophylla at Nokonoshima, southern Japan, based on several parameters such as wet weight, thallus length, size class distribution based on thallus length and reproductive types, and assessed the effect of the factors on its population fluctuation throughout the year.

MATERIAL AND METHODS

Study site and sampling

This study was carried out at Nokonoshima Island (33º36'55.04"N, 130º18'47.44"E) in Hakata Bay, Fukuoka, southern Japan (Fig. 1). The study site is characterized by the formation of rocky coral reefs that emerge in low tides (exposure for 3–4 hours at each low tide). The bottom is composed of sand, muddy–sand sediment and small stones, which serve as substrate to some fifty seaweed species (Kawaguchi, personal observations). At the study site located in the southern part of the island, *G. vermiculophylla* occupies intertidal rocky reefs below the mean tide level. The plants grew on the pebbles or on the rocks slightly covered by muddy sand.

From November 2008 to April 2010, five plants of *G. vermiculophylla* were randomly collected from the holdfast. After collection, the wet weight and the length of

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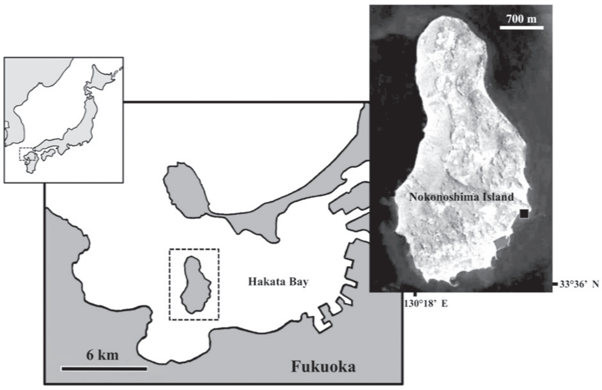

Fig. 1. Map showing the study site on Nokonoshima Island, Hakata Bay, southern Japan.

Table 1. Size class distribution of thallus length of *G. vermiculophylla*

Size class	Corresponding range of length (cm)
I	0.0 – 4.9
II	5.0 – 9.9
III	10.0 – 14.9
IV	15.0 – 19.9
V	20.0 – 24.9
VI	25.0 – 29.9
VII	30.0 – 34.9
VIII	35.0 – 39.9
IX	40.0 – 44.9

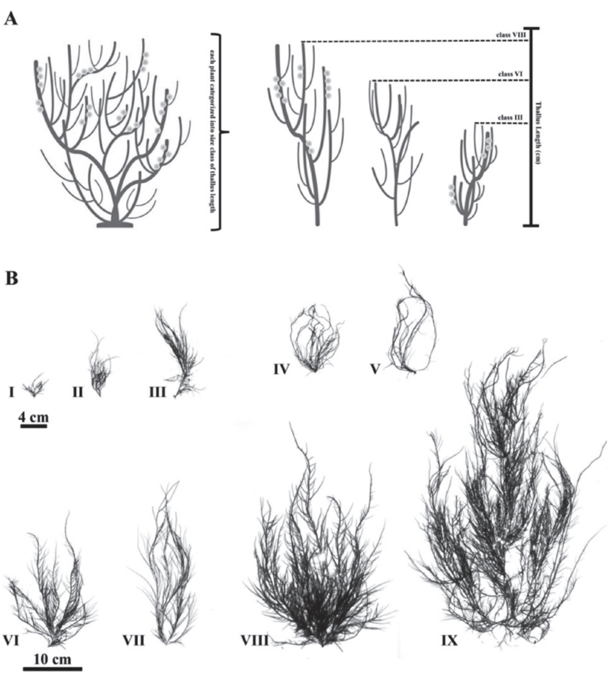

Fig. 2. Schematic diagram of measuring of thallus length and the thalli of I-IX size classes categorized on their length.

each plant were measured. For the size class distribution, the erect thalli (7–24 thalli/plant) were separated from the holdfast, and then each thallus was categorized into one of the nine size classes based on the length (Table 1, Fig. 2). The thalli that appeared shortened due to unnatural causes were excluded from the calculation. The numbers of the thalli in each size class were counted every month. Simultaneously, the reproductive types of the plants were examined by microscopic observations on their reproductive structures (spermatangial conceptacles, cystocarps or tretrasporangia) and the numbers were counted. To know the relationship between the wet weights and the thallus lengths the correlation was statistically analyzed.

Additional samplings were made throughout the year from May 2009 to April 2010 to examine the proportion of the three types of plants. Monthly, a total of one hundred thalli from the different plants were used for the microscopic observations, and the numbers of each type were counted. Seawater temperature was measured simultaneously at the collection. Other data on environmental factors, such as salinity and rainfall, were provided by the Fukuoka City Institute for Hygiene and the Environment.

Statistical analyses

All data for statistical analysis were tested initially for normality and homogeneity (Sokal and Rohlf, 1995). A one–way analysis of variance (ANOVA) was calculated to search for variations in wet weight and thallus length during the sampling period. Seasonal fluctuations of the wet weight, thallus length, and the formation of reproductive structures were compared with environmental factors such as seawater temperature, salinity, and rainfall using stepwise multiple regressions. All statistical tests and analyses were performed using SPSS statistic software version 16 (SPSS Inc., Chicago, IL, USA).

RESULTS

All environmental factors varied throughout the study period. Seawater temperature ranged from 9.5°Cin February 2009 (winter) to 26.5°C in September 2009 (summer), and the average temperature was 16.3±4.6°C. Salinity ranged from 29.8 psu in November 2009 to 32.4 psu in June 2009. The monthly rainfall was highly variable, ranging from 52.3 mm in December 2008 to 272.1 mm in August 2009; the monthly mean was 119.4±66 mm (Fig. 3).

The mean wet weight and the mean thallus length of *G. vermiculophylla* fluctuated throughout the study period: the mean wet weights (± SD) were largest from April to June, ranging from 0.41±0.01 to 0.50±0.05 g, and the largest weight was recorded in May 2009; the mean wet weights were smallest in October and November, with the smallest value of 0.009±0.002 g in October 2009; the mean thallus lengths were largest from April to June, ranging from 25.87±2.01 to 31.68±4.09 cm., and the maximum was recorded in May 2009; the mean thallus lengths were smallest in October and November, with

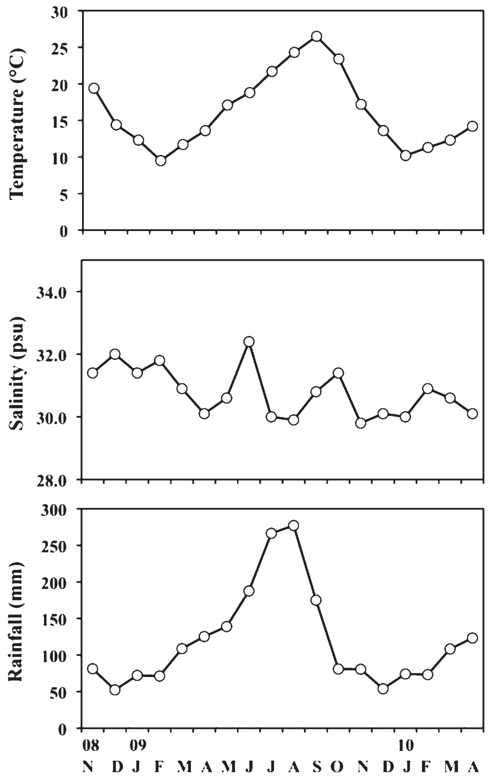

Fig. 3. Water temperature, salinity and rainfall at Nokonoshima Island, southern Japan, from November 2008 to April 2010.

the lowest value of 3.14±1.01 cm in October 2009 (Fig. 4). Additionally, the analysis of the wet weight and the thallus length showed their significant variance (Table 2). The wet weight and the thallus length were significantly and positively correlated, with a correlation coefficient of 0.96 (Fig. 5).

The size class distribution on the thallus length of *G. vermiculophylla* population varied throughout the year: in November 2008 when we started this study, only the size classes I–III were observed and the size class I was dominant; at this time, most of the thalli in the size class II was matured; in December, the size class I decreased greatly and the size class III increased; in January 2009, the size class I completely disappeared and the size class IV appeared with most of the thalli being matured; in February, the size classes II–IV grew into the size classes IV–VI and continued their growths until May when the largest size class IX was observed; during February to May, almost all the thalli were matured; in June, the size classes VIII & IX almost disappeared and gradually larger size classes decreased until September when only the size classes II–IV were observed; in October 2009, the size class I clearly reappeared and became dominant again, although quite a small number of thalli in the size class I was already observed from June to August; afterwards, a similar fluctuation pattern of the size class distribution was observed until April 2010 (Fig. 6). In our study,

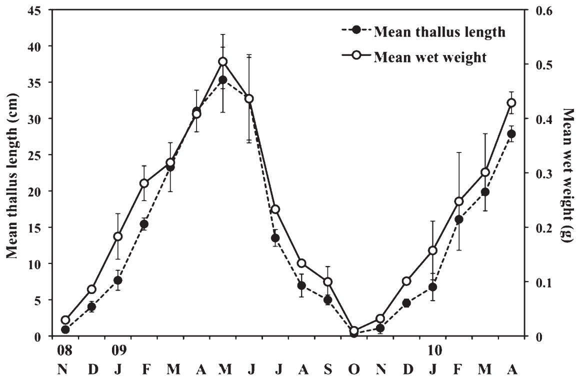

Fig. 4. Seasonal fluctuation in thallus length and wet weight of *G. vermiculophylla* from November 2008 to April 2010 (mean ± S.E.).

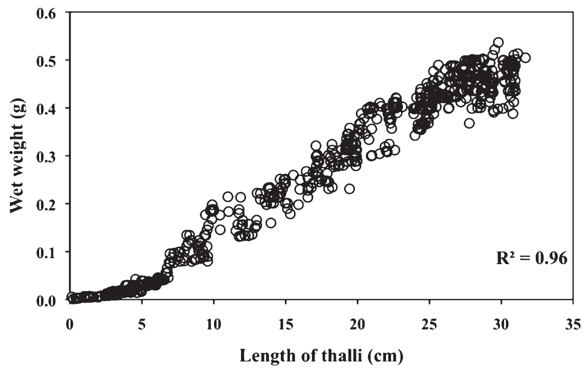

Fig. 5. Relationship between wet weight and thallus length of *G. vermiculophylla* showing positive correlation.

Table 2. One-way ANOVA calculated for wet eight and length of thalli for *G. vermiculophylla* during the sampling period

Source of variation	DF	SS	MS	F	P
Wet weight					
Months	17	1.9965	0.1170	3.6263	0.0001***
Error	72	0.0026	0.0002		
Total	89	1.9991			
Length of thalli					
Months	17	6234.9313	366.7613	1.1313	0.0023*
Error	72	23.3562	0.3245		
Total	89	6258.2875			

DF: degree of freedom; SS: sum of squares; MS: mean square; F: ration of variances.
* $P<0.05$
*** $P<0.001$

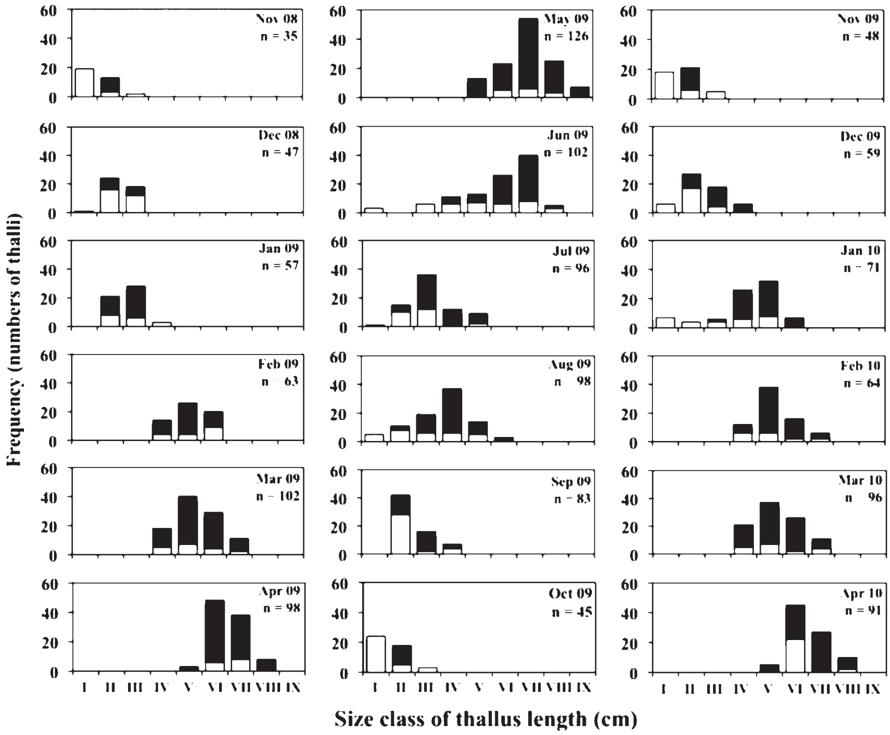

Fig. 6. Seasonal fluctuation of thallus length categorized into nine size classes in *G. vermiculophylla*. Frequency of mature thalli is shown in black.

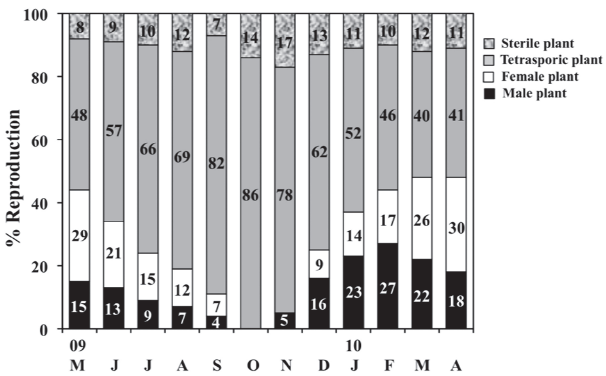

Fig. 7. Proportions of sterile and fertile plants of *G. vermiculophylla* throughout the year (May 2009 to April 2010).

reproductive structures were firstly found on the thalli in the size classe II, with the smallest sizes of the three types of thalli being 5.5 cm (tetrasporangial), 5.2 cm (spermatangial) and 6.0 cm (cystocarpic), respectively.

During one year from May 2009 to April 2010, the proportions of the three types of reproductive thalli of *G. vermiculophylla* also showed a seasonal fluctuation; the reproductive thalli showed a maximum value of 93% in September 2009 and a minimum value of 83% in November 2009; the tetrasporangial thalli were found every month with the maximum percentage of 86% in October 2009 when neither male nor female thalli were observed, and the minimum percentage of 40% in March 2010; except for March and April 2010, the tetrasporangial thalli were more numerous than sexual thalli excluding sterile ones; in the sexual thalli, the proportion of the female (cystocarpic) and the male (spematangial) thalli differed throughout the year, and the former was more

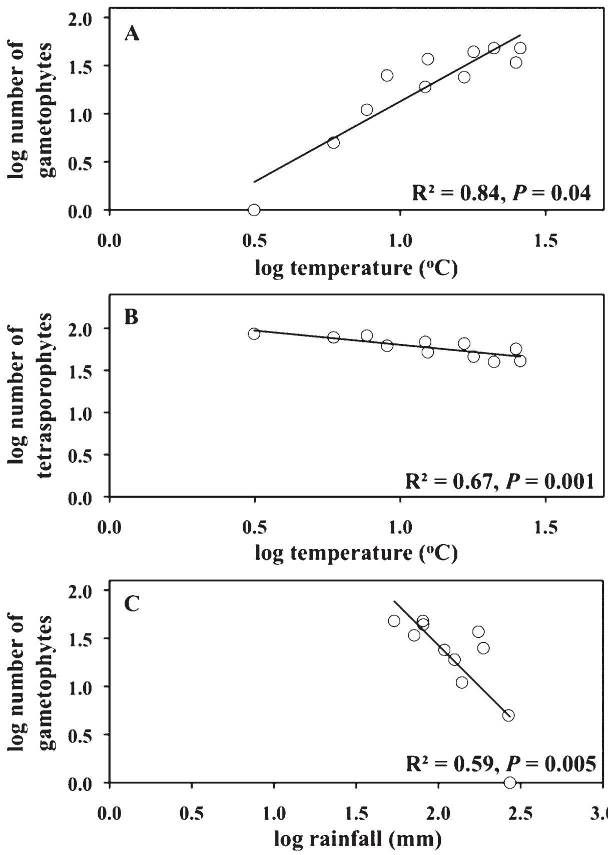

Fig. 8. Significant correlation between temperature and (A) number of gametophyte and (B) tetrasporophytes, and (C) significant correlation between rainfall and number of gametophytes.

numerous than the latter in spring and summer (from March to September 2009 and from March to April 2010); only in winter (from December 2009 to February 2010) the male thalli were more numerous than the female ones; the highest percentage (27%) of male thalli were recorded in February 2010, while the highest percentage (30%) of female ones in April 2010 (Fig. 7).

Stepwise multiple regressions showed that neither the wet weight nor the thallus length was significantly correlated with seawater temperature, salinity or rainfall ($P>0.05$). In contrast, the number of gametophytes was correlated with seawater temperature and rainfall. An increase of seawater temperature correlated to a production of gametophytes (Fig. 8A) while high rainfall resulted in a decline in the numbers of gametophytes (Fig. 8C). Furthermore, high temperature significantly correlated to a decrease of tetrasporophytes (Fig. 8B).

DISCUSSION

The population of *G. vermiculophylla* at Nokonoshima Island showed a remarkable seasonal fluctuation in growth throughout the year. The mean wet weight and the mean thallus length gradually increased from November and reached a peak in May when the maximum value was recorded, and afterwards decreased until October when the minimum value was recorded (Fig. 4). Similar patterns of fluctuation have been reported for *G. vermiculophylla* from Yamada Bay, northeastern Japan (Phooprong, 2005) and Gracilaria sp. from Tosa Bay, southern Japan (Chirapart and Ohno, 1993). In contrast, Terada *et al*. (2000) reported that the seasonal peak in biomass of *G. vermiculophylla* from Hakodate, Hokkaido, northern Japan occurred in July. This difference in the growth peak between Nokonoshima and Hokkaido populations of this alga is probably due to the difference in the fluctuation pattern of seawater temperature, as the rise of seawater temperature usually delays for a few months in northern part of Japan.

As the wet weight and the thallus length were significantly and positively correlated with a correlation coefficient of 0.96, the size classes were considered to represent developmental stages of this alga. From the size class distribution, it is supposed that new plants appear in early winter (October or November) at Nokonoshima Island because, at this period, only small thalli in the size classes Ⅰ–Ⅲ were recognized with the size class Ⅰ being dominant, and also because the mean thallus length and wet weight was the lowest (Fig. 6). The newly appeared thalli afterwards continued to grow to make a growth peak in May only when the largest size class Ⅸ was found. Our findings conform to the study of Terada *et al*. (2000), indicating that the population of *G. vermiculophylla* recruited in winter. From June to August 2009, however, a small number of thalli in the size class Ⅰ was found at Nokonoshima Island. We didn't know whether these thalli contributed to the re–establishment of the population, but considering their growth from the bases of fully grown–up plants, they might be adventitious not contributing to the population renewal.

In this study, we confirmed that the reproductive structures were formed on the small thalli in the size class Ⅱ (5.0–9.9cm). This means that *G. vermiculophylla* matures rapidly and can produce spores for a long period within the Nokonoshima population. In fact, mature plants of *G. vermiculophylla* were found throughout the year. In the meantime, Terada *et al*. (2000) reported that Hakodate population of *G. vermiculophylla* matured only from June to November and remained sterile in other months, a different pattern from that of Nokonoshima population. However, considering the similar range of seawater temperature where *G. vermiculophylla* matures at Hakodate (10–25°C) and our study site (9.5–26.5°C), it is possible to find the mature plants throughout the year at Nokonoshima Island.

Among the mature plants of *G. vermiculphylla* found at Nokonoshima Island, the asexual (tetrasporangial) plants were more numerous than sexual ones throughout the year except for March and April (Fig. 7). This is the case with some other species of *Gracilaria* (Holye, 1978; Chirapart *et al*., 1992; Terada *et al*., 2000; Phooprong, 2005) and with other red algae such as *Gelidium pusillum* (Pratthep *et al*., 2009), *Mazzaella orgegona* (Mudge and Scrosati, 2003) or *Pterocladiella capillacea* (Bottalico *et al*., 2008). In the early period of population renewal at Nokonoshima (October to December), asexual plants were dominant, but afterwards gradually sexual plants increased and exceeded asexual ones around the peak period of their growth. We have no exact explanation for this change, but it is most probable that tetrasporangial plants can mature rapidly while sexual plants, particularly female plants need much time to do so. As for this aspect of population dynamics, further studies would be apparently necessary.

In several *Gracilaria* species, the ratio of male and female plants has been reported to be nearly 1:1(Jones, 1959; Hoyle, 1978; Hay and Norris, 1984; Nelson, 1989; Chirapart *et al*., 1992). On the contrary, other studies have reported fewer female than male plants in *G. vermiculophylla* (Terada *et al*., 2000; Phooprong, 2005) and in other *Gracilaria species* (Hoyle, 1978; Engel *et al*., 2001). In our study, during May 2009 and April 2010, the ratio of male and female plants greatly fluctuated: in October 2009, when the renewal of the population started, neither male nor female plants were found; in November only male appeared; in December male increased and female first appeared; in February male reached a peak while female increased until April when the latter exceeded the former (Fig. 7). Without the fact that the tetraspores of *G. vermiculophylla* from Hokkaido developed into 1:1 male and female plants in laboratory culture (Terada *et al*., 2000), it seems reasonable to consider that the proportion of male and female plants is 1:1 in the Nokonoshima population. The monthly fluctuation in the ratio of male and female plants is very superficial, and is probably due to the difference of time necessary for the development. In fact, in March 2010 when the sexual plants are most abundant, the ratio of male and female plants was nearly 1:1.

Our study indicated that both seawater temperature and rainfall had influence on the reproduction of *G. vermiculophylla*. The rise of seawater temperature was correlated with the increase of gametophytes in this species, and at the same time with the decrease of tetrasporophytes. Yokoya *et al*. (1999) demonstrated that the highest growth rate of female *G. vermiculophylla* from Shikoku, southern Japan was observed at 25 °C and that of tetrasporophytes at 20 °C. Our observations seems to be consistent with the results. In addition, rainfall might have affected on the decrease of gametophytes. Although salinity was not significantly correlated with the growth of *G. vermiculophylla* in this study, the peaks in both mean wet weight and thallus length occurred when salinity values were high (31.5 psu). Chirapart *et al*. (1992) have also reported that mature plants of *Gracilaria changii* (Xia et Abbott) Abbott, Zhang et Xia were largely found at the places where salinity was relatively high. Salinity might be another factor affecting the reproduction in gracilarioid algae. It is not easy to specify the environmental factors controlling the seaweed population fluctuations in nature. For this, studies covering a much longer period would be needed.

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