Jpn. J. Phycol. (Sôrui) 36: 231–236, September 20, 1988

Additional notes on the life history of *Nemalion vermiculare* SURINGAR (Nemaliales, Rhodophyta)

Michio MASUDA and Kyo HORIUCHI*

Department of Botany, Faculty of Science, Hokkaido University, Sapporo, 060 Japan

MASUDA, M. and HORIUCHI, K. 1988. Additional notes on the life history of *Nemalion vermiculare* SURINGAR (Nemaliales, Rhodophyta). Jpn. J. Phycol. 36: 231–236.

Further investigations into the life history of the red alga *Nemalion vermiculare* SURINGAR were conducted at Oshoro Bay on the west coast of Hokkaido. The appearance, abundance and size of the upright gametophytes were related to tidal height and the degree of wave exposure. During the autumn and winter months, acrochaetioid filaments were found growing on barnacles on which *N. vermiculare* gametophytes grew in the summer. Sterile fragments of these filaments cultured in laboratory formed tetrasporangia under a short day regime at 20°C. Tetraspores germinated to produce a filamentous prostrate system with upright, multiaxial, terete axes that were similar to young plants of field-collected *N. vermiculare* gametophytes.

*Key Index Words: filamentous tetrasporophyte—life history—Nemaliales—*Nemalion—N. vermiculare*—Rhodophyta.*

Culture studies have demonstrated that the life history of *Nemalion vermiculare* SURINGAR (Nemaliales, Rhodophyta) comprises the alternation of a multiaxial, upright gametophyte with an acrochaetioid tetrasporophyte (UMEZAKI 1967, MASUDA and UMEZAKI 1977), as has been shown in other species in this genus (FRIES 1967, 1969, CHEN *et al.* 1978). However, the tetrasporophyte has never been observed in nature. In this paper, we report the occurrence of the tetrasporophyte in nature and present culture studies that confirm it as a phase in the life history of *N. vermiculare.* We relate patterns in the growth and reproduction of the gametophyte to its environment.

Materials and Methods

Twelve survey sites were established at Oshoro Bay (43°13′N, 140°51′E) on the west coast of Hokkaido (Fig. 1) and field observations and samplings were made twice a month from May 1986 to October 1986 and monthly from November 1986 to February 1987. These sites were selected to represent the diverse habitats of *Nemalion vermiculare* at Oshoro Bay: (1) A, C, G and L, below the low watermark and fully wave-exposed; (2) J, near the low watermark and moderately wave-exposed; (3) B, E, H and K, above the low watermark and in shallow tide pools made by large, breaking waves; and (4) D, F and I, above the low watermark and sheltered from wave action. Gametophytes, when present, and several substrates such as barnacles (*Semibalanus cariosus* and *Chthamalus challengeri*), mussels (*Mytilus edulis* and *Septifer virgatus*), perennial algae (*Sargassum thunbergii, Carpopeltis affinis* and *Chondrus pinnulatus*) and rock fragments were collected.

Culture experiments were conducted with carpospores of *Nemalion vermiculare* and excised filaments of the putative tetrasporophytes. These were cultured according to methods similar to those described earlier

* Present address: Shirakami Elementary School, Matsumae, Hokkaido 049–15.

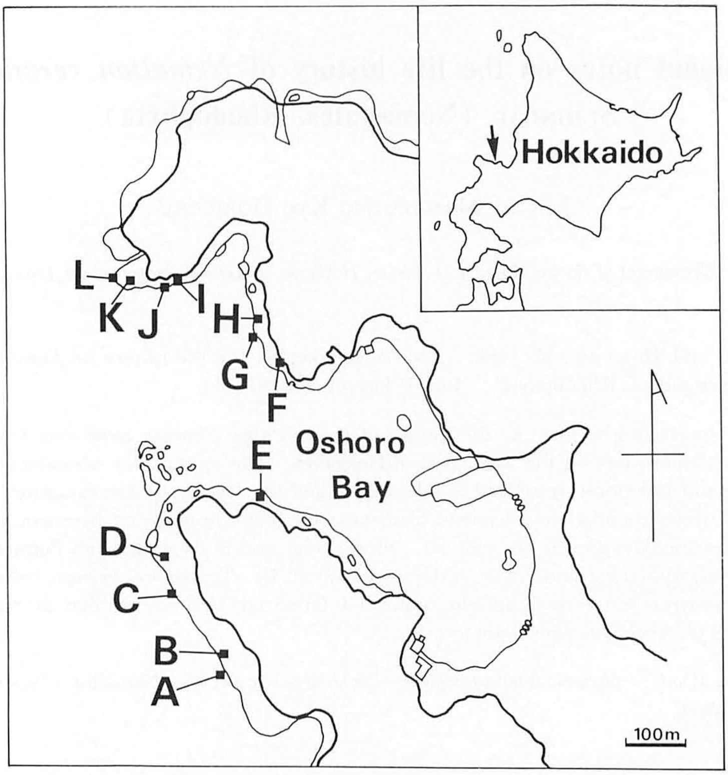


Fig. 1. Map of Oshoro Bay, showing twelve study sites.

(MASUDA and UMEZAKI 1977). The temperatures and photoperiods were regulated as follows: 5°C, 16:8 h LD (light and dark cycle); 5°C, 8:16 h LD; 10°C, 16:8 h LD; 10°C, 8:16 h LD; 15°C, 16:8 h LD; 15°C, 8:16 h LD; 20°C, 16:8 h LD; and 20°C, 8:16 h LD. The following materials were used for culture experiments: gametophytes collected on August 15, 1986 at site C and on August 28, 1986 at site E; and tetrasporophytes collected on October 25, 1986 and on November 25, 1986 at site B. Voucher specimens are deposited in the Herbarium of Faculty of Science, Hokkaido University, Sapporo (SAP 051052–051063).

Results

Phenology of upright gametophytes

Upright gametophytes (Fig. 2), less than 1 mm long, appeared in early May at all sites except the sheltered sites D, F and I, where they appeared about one month later. These young upright thalli were superficially similar to young plants of

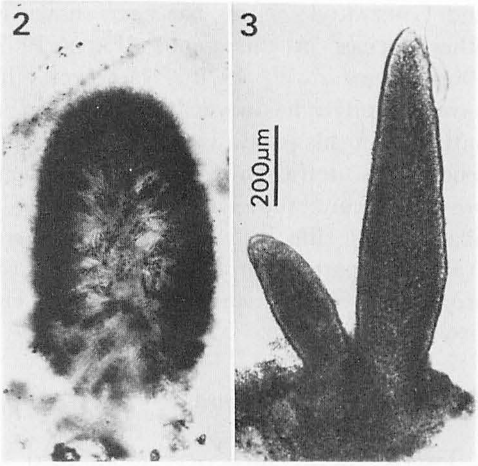
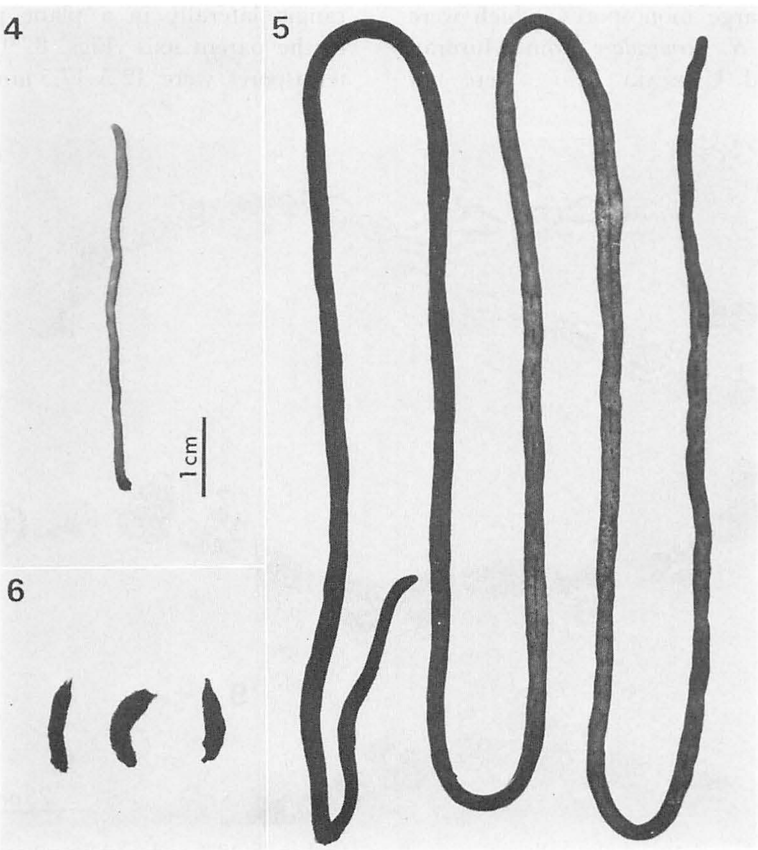


Fig. 2. Young upright thallus of *Nemalion vermiculare* collected at site C on May 9, 1986. Fig. 3. Young plant of *Gloiopeltis furcata* collected at site C on May 9, 1986. Scale in Fig. 3 applies also to Fig. 2.

Gloiopeltis furcata (Fig. 3), but *Nemalion* thalli could be distinguished from the latter by their soft, mucilaginous texture and rounded apices.

Upright thalli grew rapidly and reached maximum lengths during July and August. The number of *Nemalion* plants varied according to survey sites. The *Nemalion* plants grew abundantly at fully wave-exposed sites A, C, G and L, whereas they were less frequent at other sites. The size of upright thalli also varied according to site (Figs. 4, 5). The maximum length of thalli growing at fully wave-exposed sites was 56 cm (Fig. 5) and ten times longer than that of plants growing in shallow tide pools (Fig. 4). Plants characteristic of wave-exposed sites were thick and dark red in color.

Reproductive structures were not found on upright thalli collected in May. In mid-June, plants proved to be monoecious gametophytes, bearing both spermatangia and carpogonia near their apices. In late June, cystocarps developed at the apices while spermatangia and carpogonia continued to be produced basipetally. Assimilatory filaments constituting the cortices of fertile areas disintegrated after carpospore discharge, leaving axial filaments that also eventually eroded. Although plants declined in length as the season progressed, cystocarps continued to develop in proximal portions. In late October through November, plants only 1 cm in length (Fig. 6) formed cystocarps near their holdfasts.



Figs. 4–6. Upright gametophytes of *Nemalion vermiculare*: 4, mature plant growing in a shallow tide pool (site K) and collected on July 29, 1986; 5, mature plant growing at a fully wave-exposed place (site L) and collected on July 29, 1986; 6, old plants growing at a fully wave-exposed place (site C) and collected on October 25, 1986. Scale in Fig. 4 applies also to Figs. 5 and 6.

Culture experiments with carpospores

Isolated carpospores, 12.5–17.5 μm in diameter, were incubated under the full range of culture conditions described in Materials and Methods. They germinated and grew into acrochaetioid plants under all conditions. The plants formed tetrasporangia in a manner similar to that reported for *N. vermiculare* from Muroran (MASUDA and UMEZAKI 1977). Plants grown at 20°C, 8:16 h LD began to form tetrasporangia 12 days after inoculation, those grown at 15°C, 8:16 h LD formed tetrasporangia 14 days after inoculation, those grown at 10°C, 8:16 h LD 35 days after inoculation and those grown at 5°C, 8:16 h LD 70 days after inoculation. Liberated tetraspores were 12.5–17.5 μm in diameter. Large monospores, which were reported for *N. vermiculare* from Muroran (MASUDA and UMEZAKI 1977), were not observed. Plants grown under long day regimes did not produce tetrasporangia at any of the experimental temperatures.

Naturally occurring tetrasporophytes

Substrates (listed in Materials and Methods) were collected at the survey sites and examined under a dissecting microscope in the laboratory. From October 1986 to February 1987, sterile filaments of an acrochaetioid alga resembling the cultured tetrasporophyte of *Nemalion vermiculare* (MASUDA and UMEZAKI 1977) were found in the grooves between the longitudinal ribs of barnacle plates (Fig. 7). Excised filaments were cultured at 20°C, 16: 8 h LD for 2 weeks, and transferred to 20°C, 8:16 h LD to induce tetrasporogenesis. After 2 weeks, filaments began to produce sessile tetrasporangia laterally in a plane perpendicular to the parent axis (Figs. 8, 9). Liberated tetraspores were 12.5–17.5 μm in diameter

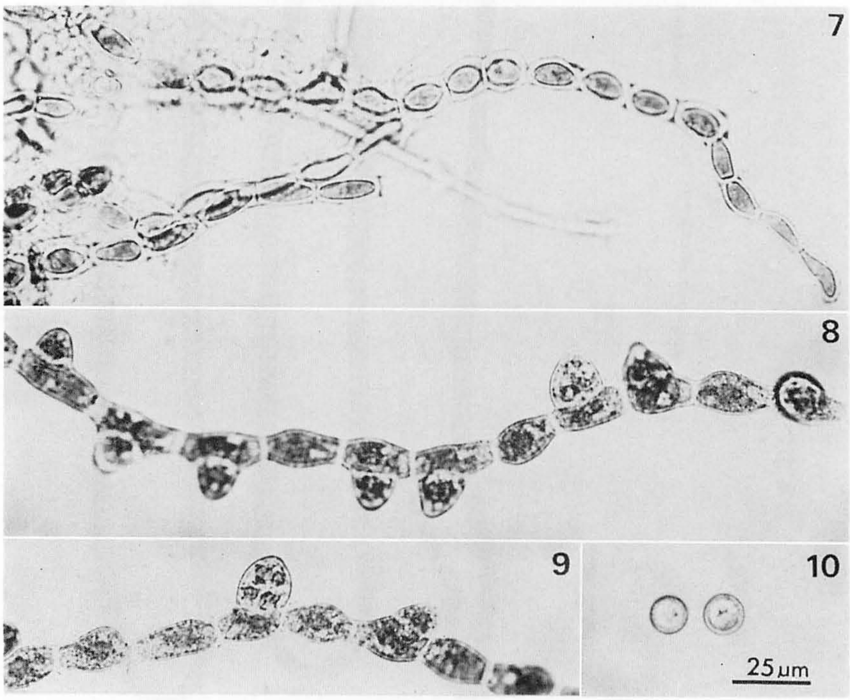
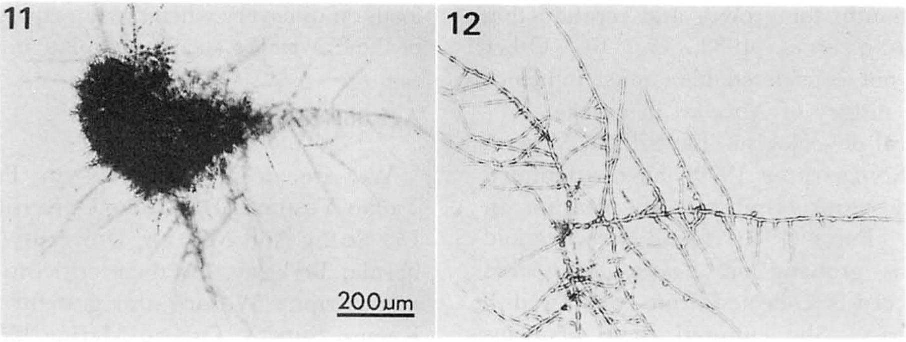


Fig. 7. Acrochaetioid alga collected at site B on October 25, 1986 and used for culture experiments [microphotographed from a specimen stained with 0.5% (w/v) cotton blue in a lactic acid/phenol/glycerol/water (1:1:1:1) solution]. Figs. 8, 9. Cultured fertile tetrasporophyte of *Nemalion vermiculare* derived from the acrochaetioid alga shown in Fig. 7. Fig. 10. Two tetraspores released from the plant shown in Figs. 8 and 9. Scale in Fig. 10 applies also to Figs. 7 to 9.



Figs. 11, 12. Twenty-one-day-old gametophytes of *Nemalion vermiculare* derived from tetraspores from the plant shown in Figs. 8 and 9: 11, grown at 15°C, 16:8 h LD and bearing an upright thallus primordium; 12, grown at 15°C, 8:16 h LD. Scale in Fig. 11 applies also to Fig. 12.

(Fig. 10). Large monospores were not observed.

Laboratory-induced tetraspores from field-collected sporophytes were cultured at 15°C, 16:8 h LD, 15°C, 8:16 h LD, 10°C, 16:8 h LD and 10°C, 8:16 h LD. Primordia of upright thalli appeared at 15°C, 16:8 h LD (Fig. 11) and 10°C, 16:8 h LD 3 weeks after spore inoculation. Each primordium consisted of a compact group of filaments arising from the center of a system of prostrate filaments. Each developed into a multiaxial *Nemalion* thallus (Figs. 13, 14). However, upright thalli did not develop at 15°C, 8:16 h LD (Fig. 12) and 10°C, 8:16 h LD.

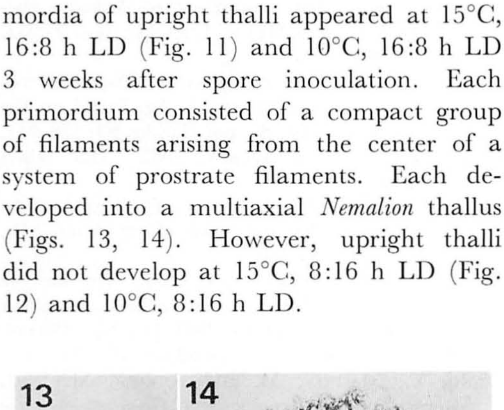


Fig. 13. Six-month-old gametophyte of *Nemalion vermiculare* derived from a tetraspore from the plant shown in Figs. 8 and 9 and grown at 15°C, 16:8 h LD. Fig. 14. Cross section of the upright thallus shown in Fig. 13.

Discussion

Our surveys show that populations of *Nemalion vermiculare* at Oshoro Bay attain their maximum abundance and size at wave-exposed localities from May through August. The summer occurrence of *N. vermiculare* gametophytes is consistent with the results of our culture experiments which indicate that upright thalli develop only under long day conditions. The tidal height has a narrow range along the coast of the Sea of Japan, but the seasonal change is large: the mean sea level is low in winter and high in summer (Japan Meteorological Agency 1985). The monthly mean sea level at Otaru near Oshoro Bay is the lowest in March (9.3 cm) and the highest in August (29.1 cm). It reaches 21.0 cm high in June (Japan Meteorological Agency 1985). This increase may account for the delayed appearance of macroscopic gametophytes in sheltered localities near the high watermark.

Under short day regimes at 5–20°C, cultured tetrasporophytes grow quickly, becoming visible to the naked eye and reproductively mature within 10 weeks after germination. Tetrasporangia are formed more rapidly at the higher temperatures in culture. Field-collected tetrasporophytes are inconspicuous and apparently sterile during the autumn and winter, even though our culture experiments indicate that light and temperature conditions are suitable during these months for growth and reproduction (cf. OHNO *et al.* 1982, Fig. 6). Other factors not considered here must influence the life history of *Nemalion* in nature.

Several phycologists (FRIES 1969, MARTIN 1969, SÖDERSTRÖM 1970) have attempted to find tetrasporophytes of *Nemalion* in nature. FRIES (1969) found acrochaetioid filaments growing on *Balanus* shells and *Ralfsia* crusts collected from the Swedish west coast. She cultured these filaments in the laboratory and succeeded to produce tetrasporangia with the same appearance as those in her previous cultures of *Nemalion multifidum* (WEBER et MOHR) J. AGARDH (FRIES 1967). MARTIN (1969) followed carposporelings of *N. helminthoides* (VELLEY) BATTERS on the Anglesey coast of Wales during several years. These acrochaetioid carposporelings growing on linpet shells and barnacle plates formed monosporangia instead of tetrasporangia in the field. They survived in the winter and produced upright gametophytes in May. SÖDERSTRÖM (1970) suggested that tetrasporophytes of *N. multifidum* in Scandinavia are induced to form tetrasporangia by rising temperature and increasing daylength. On the basis of laboratory culture experiments CHEN *et al.* (1978) concluded that tetrasporangia can be formed under various conditions of temperature and photoperiod (10–20°C, 12:12–16:8 h LD), but that a prior excursion into winter conditions (5°C, 8:16 h LD) is necessary to induce tetrasporogenesis for the Canadian *N. helminthoides*. According to our present culture experiments and those of MASUDA and UMEZAKI (1977), such an excursion is unnecessary for the Japanese *N. vermiculare*. Further investigations should focus on discovery when the tetrasporophytes of these *Nemalion* species sporulate in nature.

Acknowledgments

We are deeply indebted to Professor Tadao YOSHIDA, Hokkaido University, and Dr. Kathy Ann MILLER, University of California, Berkeley, for their criticism of the manuscript. We are also grateful to Mr. Kazuro SHINTA, Oshoro Marine Biological Station, for his help in collecting materials; and to Dr. Shin KUBOTA, Hokkaido University, for his identification of barnacles and mussels.

References

CHEN, L.C.M., EDELSTEIN, T., BIRD, C. and YABU, H. 1978. A culture and cytological study of the life history of *Nemalion helminthoides* (Rhodophyta, Nemaliales). Proc. N. S. Inst. Sci. **28**: 191–199.

FRIES, L. 1967. The sporophyte of *Nemalion multifidum* (WEBER et MOHR) J. AG. Sv. Bot. Tidskr. **61**: 457–462.

FRIES, L. 1969. The sporophyte of *Nemalion multifidum* (WEBER et MOHR) J. Ag. found on the Swedish west coast. Sv. Bot. Tidskr. **63**: 139–141.

Japan Meteorological Agency. 1985. Tide tables for the year 1986.

MARTIN, M.T. 1969. A review of life-histories in the Nemalionales and some allied genera. Br. Phycol. J. **4**: 145–158.

MASUDA, M. and UMEZAKI, I. 1977. On the life history of *Nemalion vermiculare* SURINGAR (Rhodophyta) in culture. Bull. Jap. Soc. Phycol. **25**, suppl. (Mem. Iss. Yamada): 129–136.

OHNO, Y., MASUDA, M. and KUROGI, M. 1982. Reproductive phenology of *Gigartina pacifica-ochotensis* and *Petrocelis* (Rhodophyta) in Oshoro Bay, Hokkaido. Jap. J. Phycol. **30**: 125–133.

SÖDERSTRÖM, J. 1970. Remarks on the European species of *Nemalion*. Bot. Mar. **13**: 81–86.

UMEZAKI, I. 1967. The tetrasporophyte of *Nemalion vermiculare* Suringar. Rev. Algol., N. S. **9**: 19–24.

増田道夫・堀内　京*：紅藻ウミゾウメンの生活史についての続報

北海道西岸の忍路湾において，紅藻ウミゾウメン *Nemalion vermiculare* SURINGAR の生活史を調査した。直立配偶体の出現，生育量及び生長は生育環境，すなわち，潮位と波の影響の度合に関連していることが示された。本種の着生していたフジツボに秋から冬にかけて，アクロケチウム様糸状体が生育していた。この糸状体には四分胞子嚢の形成はみられなかった。実験室で培養された糸状体は，20℃の短日条件下で四分胞子嚢を形成した。放出された四分胞子は発芽して，ウミゾウメンの若い直立体に類似した多軸型の藻体に生長した。（060 札幌市北区北10条西8丁目　北海道大学理学部植物学教室 *現住所：049-15 北海道松前郡松前町字白神928　松前町立白神小学校）