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Effect of temperature and daylength on the life history of *Coilodesme japonica* (Dictyosiphonales, Phaeophyceae)[1)2)]

By

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Introduction

The life history of Dictyosiphonales is heteromorphic with two phases of macroscopic sporophyte and microscopic gametophyte (SAUVAGEAU 1917, LOISEAUX 1967, WYNNE and LOISEAUX 1969, PETERS and MÜLLER 1985). This type of life history is often governed by temperature and daylength. Effect of temperature and photoperiod on the life history of brown algal species is well demonstrated by TATEWAKI (1966), WYNNE (1969), RHODES and CONNELL (1973) and LÜNING (1980). In Dictyosiphonales and Chordariales, gametogenesis is governed by both temperatures and daylengths. PETERS (1987) showed that the main factors affecting gametogenesis and fertilization in Chordariales are temperature and daylength.

Coilodesme STRÖMFELT is a temperate alga, occurring in northern hemisphere only. Heteromorphic life histories in the species belonging to genus *Coilodesme* have been reported by WYNNE (1972), CHEN and EDELSTEIN (1979) and PEDERSON (1984). Recently in *Coilodesme japonica* YAMADA, a unique life hitory was demonstrated by DESHMUKHE and TATEWAKI (1993), in which somatic diplodization takes place during macrothallus development. This paper demonstrates the effect of temperatures and daylengths on the life history of *C. japonica*.

Coilodesme japonica is found growing as an obligatory epiphyte on the seaweed species such as *Cystoseira hakodatensis* (YENDO) FENSHOLT, *Sargassum confusum* C. AGARDH and occasionally on *Dictyopteris divaricata* (OKAM.) OKAMURA. The macroscopic thalli are found growing from March to July. This paper is focused on the phenology and experimental control of the life cycle and morphogenesis.

Several researchers have used different terminology for different stages of brown algal life history. The term 'macrothallus' is usually referred to as sporophyte: bearing

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1) Based on a dissertation submitted in partial fulfilment of the requirements for the degree of Doctor (D. Sc), Hokkaido University (1993).

2) Dedicated to Professors MASAKAZU TATEWAKI and TADAO YOSHIDA on the occasion of their academic retirement.

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unilocular sporangia, in the Dictyosiphonales, Chordariales and Laminariales. The microscopic filamentous haplostichous stage in the life history, bearing plurilocular sporangia is often referred to as gametophyte, plethysmothallus, protonema or microthallus (WYNNE and LOISEAUX 1976). In this paper, simple terminology and abbreviation are used as follows:

Macrothallus (MT) : polystichous macroscopic generation bearing unilocular sporangia.
Microthallus (mt) : microscopic filamentous generation with plurilocular sporangia.
PH : pheromone-like odor produced by mts.
PH^+ : strains producing pheromone-like odor.
PH^- : strains not producing pheromone-like odor.

Acknowledgements

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Materials and Methods

Culture

Macrothalli of *C. japonica* were collected growing epiphytically on *Cystosiera hakodatensis* in tide pools or in lower intertidal zones at Muroran (June 1989, May 1990 and 1992) and Akkeshi (May 1990 and April 1992) in Hokkaido, Japan as shown in Table 2.

Cultures were initiated from zoospores liberated from unilocular sporangia. For isolating strains, unispores were first grown in a petri dish containing 10 or 15 m*l* of $ASP_{12}NTA$ culture medium (PROVASOLI 1963) at 14°C and 14 : 10 LD. Under this condition, initiation of plurilocular sporangium formation was delayed (DESHMUKHE and TATEWAKI 1993). Strains were selected by cutting and isolating individual branches of vegetative mts of 100-200μm in diameter. Cultures were then maintained in test tubes containing 10m*l* PESI medium (TATEWAKI 1966) under conditions with different combinations of temperature and daylength (Table 1) with irradiance of 35-45μmol $m^{-2}s^{-1}$. Isolation dates and codes are described in Table 2.

Studies on pheromone-like odor

Release of pheromone-like odor was confirmed after mt strains were isolated. Only PH^+ type strains such as A-7'90, A-31'90 and M-4'90 were cultured vegetatively under condition no. 7. They were then transferred back to condition no. 3 to induce pheromone release. When the plurispores were released and settled down, the odor was detectable

organoleptically (by nose).

Five hundred m*l* of the culture medium with strong odor was transferred to an extraction vessel (1 *l*). The emanating odor was entrapped in a filter consisting of 5 mg activated carbon in a closed-loop-stripping system (GROB and ZÜRCHER 1976) for 8 h at room temperature. Volatile compounds adsorbed on the carbon were further eluted with dichloromethane (50 μl). The elute was analyzed by HPLC (Hitachi 655 A-11) instrument with a reversed phase column Zorbax ODS 4.6 mmϕ×250 mm, a mobile phase acetonitrile-water (85 : 15), at flow rate 1.0 m*l*/min, a detector at 263 nm. A major peak was separated by preparative HPLC under the same condition and the isolated compound was subjected to UV analysis (Hitachi spectrophotometer 124).

Effect of temperature and daylength

Experiments with microthalli were carried out in two different sets :

a) microthalli (mts) were first grown under condition no. 3, and then transferred to all the eleven conditions (Table 1).

and

b) mt cultures were first grown under conditions (nos. 1-5) and then transferred into condition no. 6.

The first set of experiments was to find the most favorable condition for MT development. By the second set of experiments, effect of daylength on the maturation of mt and its consequence on MT development were examined.

Table 1 Culture conditions employed for the experiments

No.	Temperature (°C)	Photoperiod Light : dark (h)
1	5	10 : 14
2	5	11 : 13
3	5	12 : 12
4	5	13 : 11
5	5	14 : 10
6	10	10 : 14
7	10	14 : 10
8	14	10 : 14
9	14	14 : 10
10	18	10 : 14
11	18	14 : 10

Critical incubation time necessary to induce MT development from germinating spores

Firstly, strains nos. A-11'90, A-31'90, M-4'90 and M-10'90 were grown vegetatively

at 10 °C, 14 : 10 LD, and transferred to 5°C, 12 : 12 LD. These strains were then transferred to 10°C, 14 : 10 LD at 5-day intervals and developmental patterns of sporelings were recorded.

Effect of temperature and daylength on the growth of MT

Macrothalli were obtained as described by DESHMUKHE and TATEWAKI (1993). When about 2 weeks old, ten fronds were transferred to each culture condition described

Table 2 Coding and origin of the strains used

Code	Origin	Isolation date
FM'89	Muroran field MT	3 June 1989
FA'90	Akkeshi field MT	9 May 1990
FM'90	Muroran field MT	19 May 1990
FM'92	Muroran field MT	28 May 1992
FA'92	Akkeshi field MT	16 April 1992
CA-26'91	Culture MT (A-26'90)	10 September 1991
CA-31'91	Culture MT (A-31'90)	15 September 1991
CM-13'91	Culture MT (M-13'90)	24 October 1991
CM-34'91	Culture MT (M-34'90)	24 October 1991
CM-4'92	Culture MT (M-4'90)	8 January 1992
CA-11'92	Culture MT (A-11'90)	15 February 1992

in Table 1. Growth was measured in terms of length at weekly intervals. All the experiments were carried out in triplicate using PESI as the culture medium.

Results

Life history

The life history of *Coilodesme japonica* is heteromorphic with alternation of generations (Fig. 1). It has two different morphological phases. One, which is found in the nature, is macroscopic and the other microscopic. Macroscopic thalli grow to 20-30 cm or sometimes more than 50 cm in length and 1.0-2.5 cm in width. Unilocular sporangia are found sunken in the cortical cells of the macrothallus. The unispores, after swimming a while, settled down and formed germination tubes within 1-2 days, and grew to prostrate filaments. At higher temperatures, uniseriate or biseriate plurilocular sporangia developed on mts within 2-3 weeks. The plurispores were similar to the unispores in morphology and germination pattern. There was no distinction among different strains. Nevertheless, no fusion was observed between spores from PH^+ and PH^- strains. However, initially at 5°C, 12 : 12 LD, pheromone-like odor was noticed in certain strains. Effects of different temperatures and daylengths on mt/MT development are shown in Table 3. Pheromone-like odor was slightly noticed in conditions no. 2 and no. 4 but was

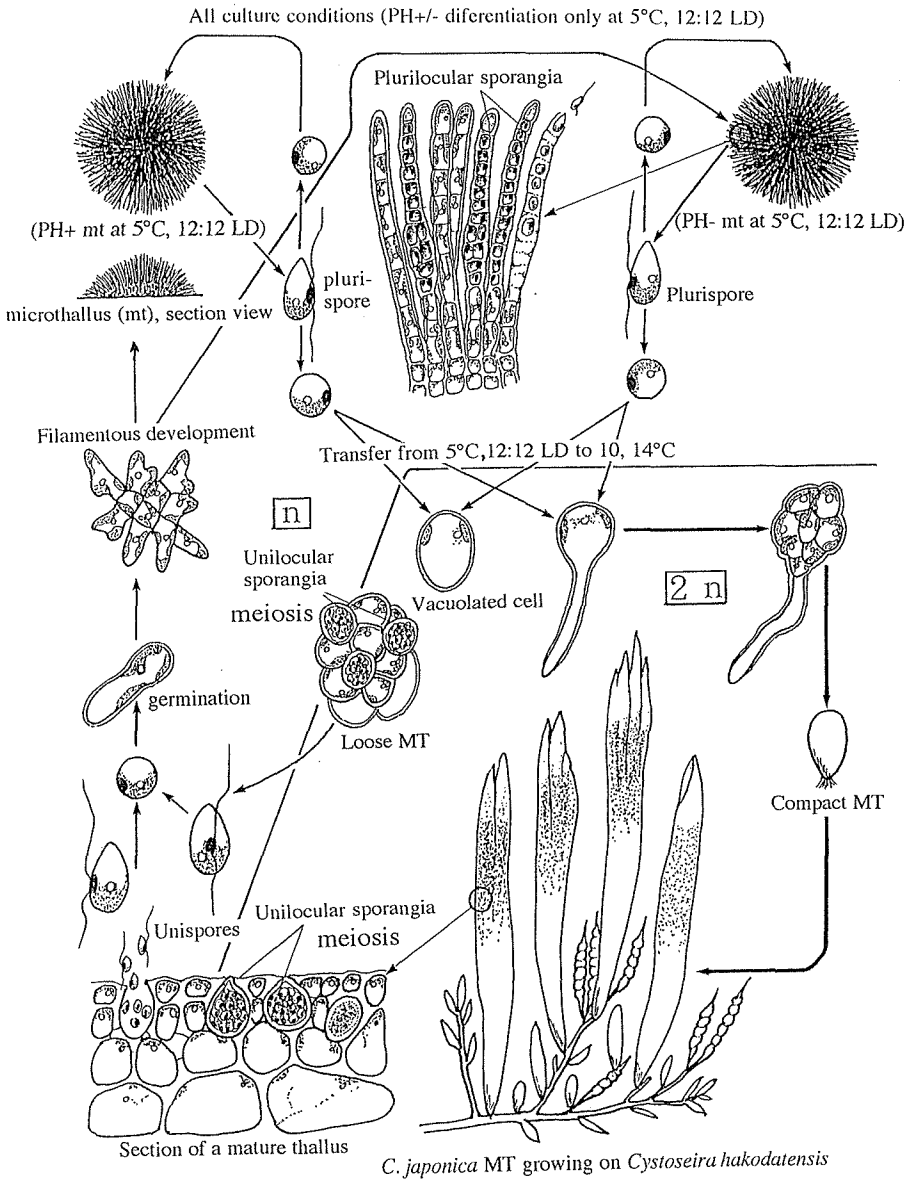

Fig. 1 Life history of *Coilodesme japonica* YAMADA

Table 3 Effect of culture conditions on the pheromone-like odor and mt/MT morphogenesis

Culture Condition No.	(°C	L : D)	Pheromone like odor	mt morphogenesis	MT morphogenesis
1	5	10 : 14	−	100	0
2	5	11 : 13	+/−	100	0
3	5	12 : 12	+++	100	0
4	5	13 : 11	+/−	100	0
5	5	14 : 10	−	100	0
6	10	10 : 14	−	100	0
7	10	14 : 10	−	100	0
8	14	10 : 14	−	100	0
9	14	14 : 10	−	100	0
10	18	10 : 14	−	100	0
11	18	14 : 10	−	100	0

+++ : strong, +/− : weak, − : none.

Table 4 Distribution of pheromone-like odor in *Coilodesme japonica* microthallus of various origin

Origin of strain	No. of microthalli		
	PH^+	PH^-	Total
FM'89	13	25	38
FA'90	22	14	36
FM'90	18	22	40
FM'92	32	25	57
FA'92	18	38	56
CA-26'91	23	15	38
CA-31'91	16	20	36
CM-13'91	17	23	40
CM-34'91	13	22	35
CM-4'92	17	22	39
CA-11'92	35	29	64

maximum in condition no. 3. The ratio between PH^+ and PH^- microthalli was almost 1 : 1 (Table 4).

Development of macrothalli

Macrothallus initiation was triggered by growing strains first at 5°C, 12 : 12 LD and then transferring to higher temperatures. Thereafter, three different types of germination patterns were observed : 1) Some plurispores germinated into filamentous mts ; 2) Some other started enlarging in the size and became vacuolated cell ; and 3) The other formed

a very long germ tube. In the second and the third types, spores did not divide up to 3 days.

In the second type, a vacuolated cell divided to form a loose mass of cells resembling to the medullary cells of macrothallus. After one month, few cortical cells developed and unilocular sporangia were formed within the cortical cells. In the third type, however, cell division occurred within 4-5 days and a compact mass of cortical tissues were formed. Within one month, it acquired a form of hollow sac and unilocular sporangia developed within 2-3 months sunken in the cortical tissues. The unispores released displayed similar development to that of unispores released from the field macrothallus.

It had been demonstrated that somatic diploidization takes place in the spore germi-

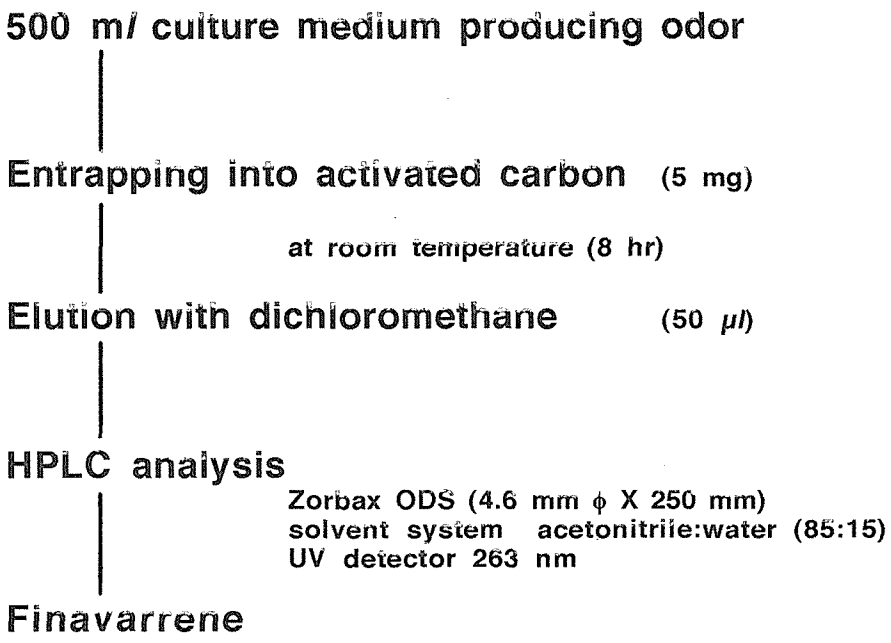

Fig. 2 Schematic identification procedure of separation of pheromone-like odor substance

nation during the development of MT (DESHMUKHE and TATEWAKI 1993).

Identification of the pheromone-like odor substance

The pheromone-like odor showed a complex HPLC profile (as shown in Figs. 2, 3), with a major peak at 8.86 min. From UV spectrum (λmax 245 (sh), 254, 263, 274 nm) and HPLC retention data of this peak, the major component was tentatively identified as (1, 3*E*, 5*Z*, 8*Z*)-undecatetraene. This derivative is commonly known as "finavarrene",

which is found as a sex pheromone in many brown algal female gametophyte (MAIER and

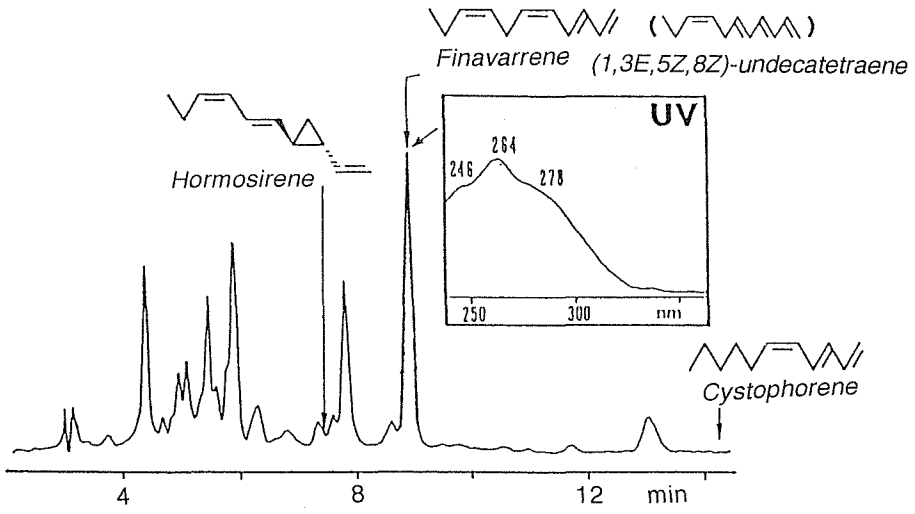

Fig. 3 Elution profile of pheromone (finavarrene) obtained from analytical HPLC
Flow rate: 1mℓ/min

MÜLLER 1986, PETERS 1987). Pheromone molecules such as (1, 3*Z*, 5*Z*)-undecatetraene (cystophorene) and *trans*-1- (1*E*, 3*Z*-hexadienyl)-2-vinylcyclopropane (hormosirene) were detectable by the HPLC at 263 nm. However, the last two were in negligible quantity.

Effect of temperature and daylength

Table 3 represents the presence or absence of pheromone-like odor and MT/mt formation in *C. japonica* strains grown in each culture condition. The odor was much intense under condition no. 3. Although pheromone-like odor was present at the same temperature with ±1 h difference in daylength, it was less intense. MT development did not take place in any condition.

Because a slight differentiation had been observed under condition no. 3, all the strains were transferred from this condition to all the conditions in the next set of experiment. The results are shown in Table 5. After the transfer, MT development was observed at higher temperatures. Maximum MT formation took place under condition no. 7 where 41.2% sporelings developed into MT. At lower temperatures, MT development did not take place.

In Table 6, effects of different photoperiods at 5°C on MT development are shown. In strains grown under 10 : 14 LD (short day condition), the odor was not detectable and these strains did not show any MT development. Similar results were observed from

Table 5 Effect of various culture conditions on MT morphogenesis (strains incubated at 5 °C, 12 : 12 LD before transfer)

Cond. No.	mt(%)	MT(%)
1	100	0
2	100	0
3	100	0
4	100	0
5	98.6	1.4
6	72.5	27.5
7	58.8	41.2
8	79.0	21.0
9	65.3	34.7
10	100	0
11	100	0

The MT/mt % is calculated based upon the germination pattern, i.e., after two weeks of spore germination.

strains grown under 14 : 10 LD (long day condition). MT development varied from strain to strain. Some strains, even after slight differentiation, did not produce any macrothalli (e. g., A-18'90). The presence or absence of the pheromone did not show much difference in MT initiation. Best pretreatment condition for the macrothallus initiation was thus 5°C, 12 : 12 LD (>50% MT formation was observed in several strains).

Critical preincubation for MT induction

Results obtained after transferring four representative strains from 5°C, 12 : 12 LD to 10°C, 14 : 10 LD are summarized in Table 7. Plurilocular sporangia were still absent after 5 days preincubation. They were formed, however, after about 4-5 days at 10°C. Obviously, the odor was absent at that time. The liberated spores germinated into microthallus. After 10-day preincubation, some of mts showed immature development of plurilocular sporangia. In most of cases where mature plurilocular sporangia were formed, spore release was observed within 2-4 days after transferring 10°C. All the spores germinated into microthalli.

Fifteen or 20-day incubation, however, showed different results. After two weeks, mature plurilocular sporangia were observed. In strain A-31'90, spore release was observed at the time of transfer and the pheromone-like odor was noticeable. Although spores were not released in some strains, they were released within a day after transfer. Percentage of macrothallus formation varied from 28.1% (in A-11'90) to 56.4% (in A-31'90,

Table 6 MT morphogenesis after pretreatment under various photoperiods of 5 °C

Strain No.	10:14		11:13		12:12		13:11	
	mt	MT	mt	MT	mt	MT	mt	MT(%)
A-1'90(PH$^-$)	100	0	78.4	21.6	75.9	24.1	90.2	9.8
A-5'90(PH$^+$)	100	0	100	0	100	0	100	0
A-7'90(PH$^-$)	100	0	83.9	16.1	78.6	21.4	100	0
A-11'90(PH$^-$)	100	0	51.1	49.9	43.4	56.6	61.0	39.0
A-18'90(PH$^+$)	100	0	100	0	100	0	100	0
A-23'90(PH$^-$)	100	0	100	0	100	0	100	0
A-26'90(PH$^+$)	100	0	55.4	44.6	46.5	53.5	68.5	32.5
A-31'90(PH$^+$)	100	0	60.5	39.5	47.8	52.2	55.6	44.4
A-34'90(PH$^-$)	100	0	100	0	100	0	100	0
A-40'90(PH$^+$)	100	0	65.8	34.2	70.8	29.2	100	0
M-1'90(PH$^+$)	100	0	57.0	43.0	36.0	64.0	68.2	31.8
M-4'90(PH$^+$)	100	0	63.6	36.4	38.6	61.4	62.8	37.2
M-8'90(PH$^-$)	100	0	78.4	21.6	75.9	24.1	90.2	9.8
M-10'90(PH$^-$)	100	0	100	0	50.6	49.4	100	0
M-13'90(PH$^+$)	100	0	100	0	77.3	22.7	100	0
M-19'90(PH$^+$)	100	0	100	0	100	0	100	0
M-21'90(PH$^-$)	100	0	94.4	5.6	83.0	17.0	100	0
M-24'90(PH$^-$)	100	0	86.8	13.2	72.3	27.7	85.7	14.3
M-32'90(PH$^-$)	100	0	100	0	100	0	100	0
M-34'90(PH$^+$)	100	0	57.3	42.7	45.1	54.9	100	0

The MT/mt % is calculated based upon the germination pattern, i. e., after two weeks of spore germination.

15-day incubation). Plurispore release was observed in all the strains after 20-days of preincubation. The pheromone-like odor was noticeable in PH$^+$ strains. A percentage of macrothallus formation slightly increased, but there was no significant difference. The maximum percentage of MT formation was observed A-31'90 strain (61.4%). The macrothallus formation was observed in only first set of spore release. The spores which were released after 3-5 days in 10°C, germinated in microthalli.

Effect of temperature and daylength on MT growth

Results of various temperatures and photoperiods for MT growth are shown in Figure 4. At lower temperatures, growth was inhibited and thalli never grew more than 0.1 mm in length. Under these conditions, unilocular sporangia did not develop. Again the most suitable condition was 10°C, 14 : 10 LD, where within one month, thalli grew up to 2 mm. At other temperatures, i. e. 14 and 18°C, MT growth was better in long than short daylength. At 18°C, growth was suppressed but unilocular sporangia developed on the thalli.

Tables 7 Critical preincubation period (at 5°C, 12:12) required for mt strains to produce MT from germinating spores

Srain No.	Pl(days)	Ps	PH	mt(%)	MT(%)
5-days incubation					
A-11'90(PH$^-$)	−(3-4)	5		100	0
A-31'90(PH$^+$)	−(5)	7	−	100	0
M-4'90(PH$^+$)	−(4)	5-6	−	100	0
M-10'90(PH$^-$)	−(3)	5-6		100	0
10-days incubation					
A-11'90(PH$^-$)	+	2		100	0
A-31'90(PH$^+$)	−(2)	4	−	100	0
M-4'90(PH$^+$)	+	1	−	100	0
M-10'90(PH$^-$)	+	2		100	0
15-days incubation					
A-11'90(PH$^-$)	+	1(3)		71.9(100)	28.1(0)
A-31'90(PH$^+$)	+	2(3)	+(−)	43.6(100)	56.4(0)
M-4'90(PH$^+$)	+	1(4)	+(−)	65.5(100)	34.5(0)
M-10'90(PH$^-$)	+	1(4)		67.8(100)	32.2(0)
20-days incubation					
A-11'90(PH$^-$)	+	0(4)		48.5(100)	51.5(0)
A-31'90(PH$^+$)	+	0(5)	+(−)	38.6(100)	61.4(0)
M-4'90(PH$^+$)	+	0(3)	+(−)	42.8(100)	57.2(0)
M-10'90(PH$^-$)	+	0(3)		60.0(100)	40.0(0)

Pl: formation of plurilocular sporangia
Ps: plurispore released in no. of days after transfer
PH: pheromone-like odor; mt: microthallus; MT: macrothallus
Bracketed figures indicate continuous culture period at 10°C, 14: 10 condition, and percentage of mt and MT development

Discussion

The present study positively showed that the life history of *Coilodesme japonica* is governed by both temperature and photoperiod. The plurispores were unable to undergo morphogenetical changes into macrothallus without the following changes of culture conditions. Strains were treated with 5°C, 12 : 12 LD and then transferred to higher temperatures. These results are consistent with the phenology of *C. japonica*. The macroscopic thalli of *C. japonica* become visible in nature growing on the host plants from March onwards.

The effect of temperature and photoperiod on different stages of marine algal life history is a well-known factor, especially in brown algae. This has been proved positive-

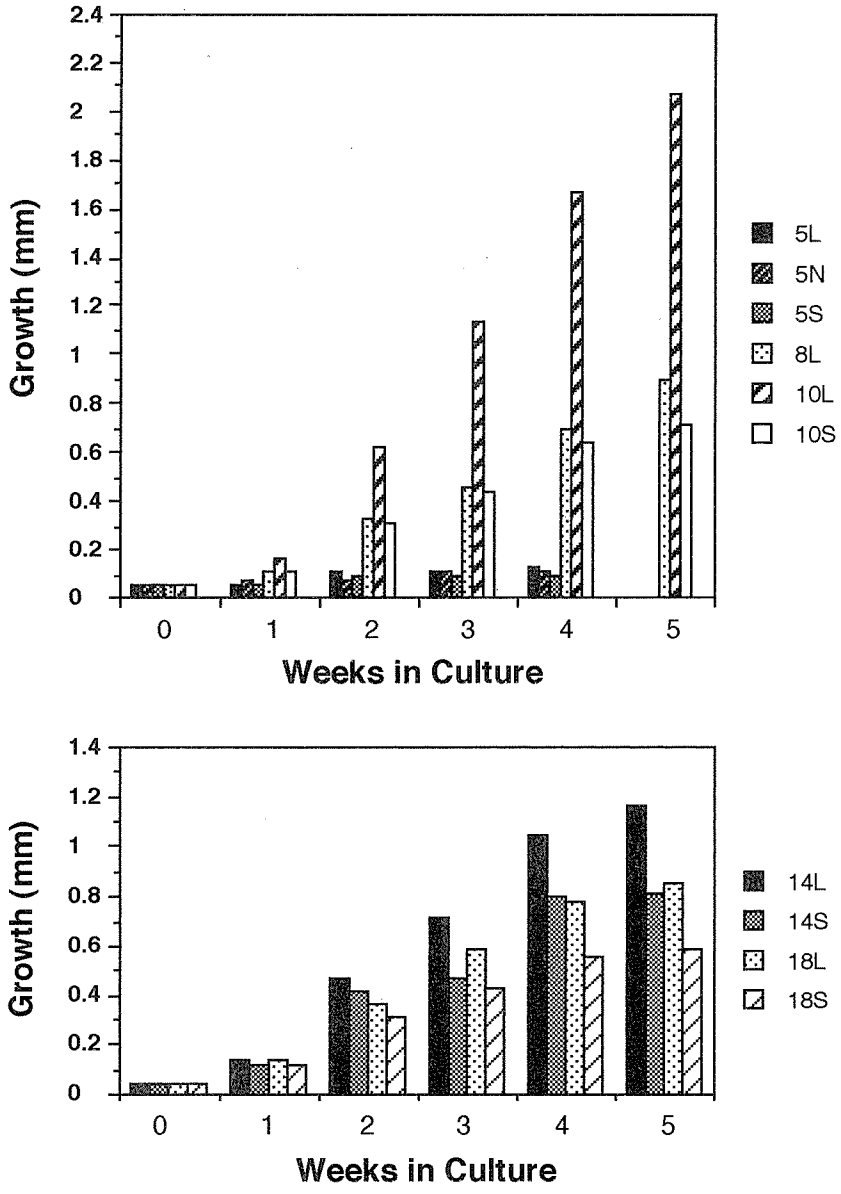

Fig. 4 Effect of various culture conditions on the growth of *Coilodesme japonica* MT

ly in *Scytosiphon* (TATEWAKI 1966, DRING and LÜNING 1975, NAKAMURA and TATEWAKI 1975, CLAYTON 1976a, b, 1978, 1980), in *Colpomenia peregrina* (CLAYTON 1979) and in the Laminariales (NAKAHARA 1984). Morphogenesis in *C. japonica* took place only after transferring the strains from conditions of nos. 2, 3 or 4 to higher temperatures. Under natural conditions, the macroscopic thalli are clearly visible to the naked eye from April onwards. The microscopic filamentous stage of *C. japonica* may be present from autumn to early spring and they may produce spores that germinate into the macroscopic thalli. Although 5°C, 12 : 12 LD condition seems to be the best for MT development, small differences of ±1 hour to the neutral daylength are also suitable. In *Myriotrichia clavaeformis* (Dictyosiphonales), PETERS (1988) has demonstrated that gametogenesis occurs under low temperatures and sporophytes develop at higher temperatures.

PETERS (1987) showed that in members of Chordariales, gametogenesis is induced by lower temperatures, while at higher temperatures, the spores are not sexually differentiated. In *Dictyosiphon foeniculaceus*, the gametogenesis is induced at 5°C at both long and short day (PETERS and MÜLLER 1985), or neutral day (DESHMUKHE and TATEWAKI unpublished). The gametogenesis in such cases is associated with sex differentiation, where the female gametophyte produces a sex pheromone. In the present study, some *C. japonica* mt strains produced pheromone-like odor at 5°C, 12 : 12 LD condition. This odor, was analyzed by HPLC and identified as finavarrene. Finavarrene has been identified as a sex hormone in *Ascophyllum nodosum* (MÜLLER *et al.* 1982) and *D. foeniculaceus* (PETERS and MÜLLER 1985) and *D. hirsutus* (PETERS 1992). Other two minor components hormosierene and cystophorene are also found as sex pheromones in several brown algae (MAIER and MÜLLER 1986). In the case of *C. japonica*, however, these pheromones do not have similar functions, as neither the sexual reproduction nor gamete attraction was observed. Lack of such activities by finavarrene is also reported in some brown algae. In the case of *Spermatochnus paradoxus*, although sexual reproduction is present, pheromone does not seem to have any role (MÜLLER *et al* 1981, PETERS *et al* 1987). Apart from this, in two Chordariales species, *Papenfussiella lutea* and *Acrothrix gracilis*, despite the pheromone presence, sexual reproduction was absent (PETERS 1987). He reported that some strains produced finavarrene and others did not. Fusion between zoids of two different strains was not observed. He suggested the possibility of isolating cultures from different parents, either asexual or unisexual female. This possibility is not ruled out, but in *C. japonica*, strains isolated from a single MT, (either from PH^+ or PH^- origin) also showed PH^+ and PH^- characteristic. Therefore, it could be likely that in *C. japonica* also pheromone production may have remained as a vestigial character.

An experiment on determining the critical incubation of microthalli at 5°C, 12 : 12 LD has confirmed that minimum 15-day incubation is essential for the macrothallus development. The macrothallus formation could be achieved when the spores were liberated

within 1-2 days after transferring to higher temperature conditions from 5°C, 12 : 12 LD. But plurispores released afterwards developed into the microthallus. This means the occurrence of sexual differentiation that induces the macrothallus formation. After 5 or 10-day incubation at 5°C, 12 : 12 LD this differentiation does not occur. Also in *Dictyosiphon foeniculaceus*, sexually differentiated gametes can develop into the haploid sporophyte and asexual plurispores germinate into the microthallus (PETERS and MÜLLER 1985, PETERS 1992, DESHMUKHE and TATEWAKI unpublished). A similar situation has also been demonstrated in the Chordariales (PETERS 1987).

The growth rate was also affected by temperature and daylength. Both 10°C and 14°C long-day conditions were most preferable conditions. These results were consistent with the phenology of *Coilodesme japonica*, where the maximum growth was noted in May -June. Under field conditions, *C. japonica* MT thalli disappear from midsummer when the temperature rises above 20°C. Formation of unilocular sporangia at 18°C is also consistent with natural habitat of *C. japonica*. WYNNE and LOISEAUX (1976) in their review of brown algal life histories suggested that the reproduction and morphological phases are governed by environmental factors such as temperatures and photoperiods. Regulation of life history of *Desmotrichum undulatum* by temperature is demonstrated in detail by RIETEMA and VAN DEN HOEK (1981). They stated that in this species maximum length of thalli was observed under 4-12°C, long-day conditions that was parallel to the natural habitat. In *Acrothrix gracilis* (Chordariales) from Akkeshi (northeastern Hokkaido, Japan), erect thalli were produced at 10°C, long-day condition and unilocular sporangia developed in warmer temperatures (AJISAKA and KAWAI 1986). In this studies also, I noticed that the microthalli could be differentiated (occurring of pheromone-like odor; PH^+/PH^-) at 5°C, 12 : 12 LD (or +1 h) and the macrothalli could be obtained at 10°C, 14 : 10 LD. Interestingly the phenology of *C. japonica* also showed that the microthalli must be present from August till (February) March under field conditions. Most macrothalli start appearing from March-April. This gives microthalli a short period to become sexually (?) differentiated. This transit period in the field is more or less similar to 5°C, 12 : 12 LD. The highest number of thalli are found from late April to May (about 10°C, 14 : 10 LD). The maximum growth can be achieved at 10°C, 14 : 10 LD condition, which is parallel to the natural habitat of *C. japonica*. This means that under natural conditions also the life history of *C. japonica* is controlled by temperature and daylenght.

Summary

Coilodesme japonica is an epiphytic plant with heteromorphic alternation of generations. This alga is found along northeast coast of Japan. The diploid phase is macrothallic (MT) and the haploid phase is microthallic (mt).

Spores released from unilocular sporangia develop into filamentous microthallic plants. The results were uniform at all the 11 culture conditions. A kind of sex

differentiation (pheromone: finavarrene) was observed in 5°C, 12: 12 LD (neutral day) condition. MT morphogenesis occurred only after treating the microthalli under this condition and then transferring them into higher temperature conditions. Highest MT morphogenesis (41.2%) was observed at 10°C, 14: 10 LD (long day) followed by 14°C, 14: 10 LD. Similarly 10°C, 14: 10 LD was found favorable to the further MT growth. Unilocular sporangial development was advanced at the higher temperatures such as 14°C and 18°C.

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