

Life history and sexuality of archeospore and apogamy of *Bangia atropurpurea* (Roth) Lyngbye (Bangiales, Rhodophyta) from Fukaura and Enoshima, Japan

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ABSTRACT: Culture studies were conducted on two local populations of a marine alga *Bangia atropurpurea* (Roth) Lyngbye from Enoshima and Fukaura, Japan. The wild plants from Enoshima and Fukaura were asexual and dioecious sexual plants, respectively. The archeospore germling has the same sexuality of the parental plant of gametophytes in the dioecious Fukaura plant. Isolated male or female gametophytes produced apogamous conchocelis from the vegetative cells of each plant. These conchocelis filaments produced conchosporangial branches, and the liberated conchospores grew to gametophytes. The sexuality of the gametophyte is the same as the sexuality of the mother plant. Archeospores from Enoshima and Fukaura plant were cultured at various temperatures of 5–30°C under a photon flux density of 80 $\mu\text{mol photons/m}^2/\text{s}$ and day lengths of 14 L : 10 D and 10 L : 14 D. After 40 days in culture, the maximum plant length of Fukaura was markedly smaller than those of Enoshima at 10–25°C. The Enoshima plant produced archeospores only and grew well at 15–25°C, but did not grow at 30°C after 16 days. However, the Fukaura plant grew at 10–30°C and grew well at 20–25°C. The plant produced zygotospores at 15–20°C within 40 days in culture. The results of these observations suggest that the plants of Enoshima and Fukaura have different types of life history, and the plant of Fukaura is a completely dioecious plant with an apogamic reproductive subcycle.

KEY WORDS: apogamy, archeospore germling, *Bangia atropurpurea*, growth, life history, maturation, sexuality.

INTRODUCTION

Bangia atropurpurea (Roth) Lyngbye is distributed from cold to temperate regions of both hemispheres, and the habitat is known usually at the upper intertidal zone along marine coastlines and in freshwater.^{1–7} Asexual and sexual plants of *B. atropurpurea* were recognized in marine populations.^{6,8} In Japan, the marine form of this species is widely distributed from Hokkaido to Kyushu. Notoya and Kikuchi⁹ have reported that the life history of the plant from Banda, Chiba Prefecture has dioecious gametophytes and a clearly biphasic cycle. However, the sexuality of archeospore germlings from the male or female gametophyte has not yet been reported.

The present study aims to clarify the life history of two marine populations of asexual and sexual

plants of *B. atropurpurea* from Enoshima and Fukaura and the influence of temperature and day length on the growth and maturation of gametophytes and/or conchocelis. In particular, in the present report, new knowledge on the life history of this species, the sexuality of archeospore germlings and on conchocelis from the vegetative cells of the male and female gametophytes will be presented.

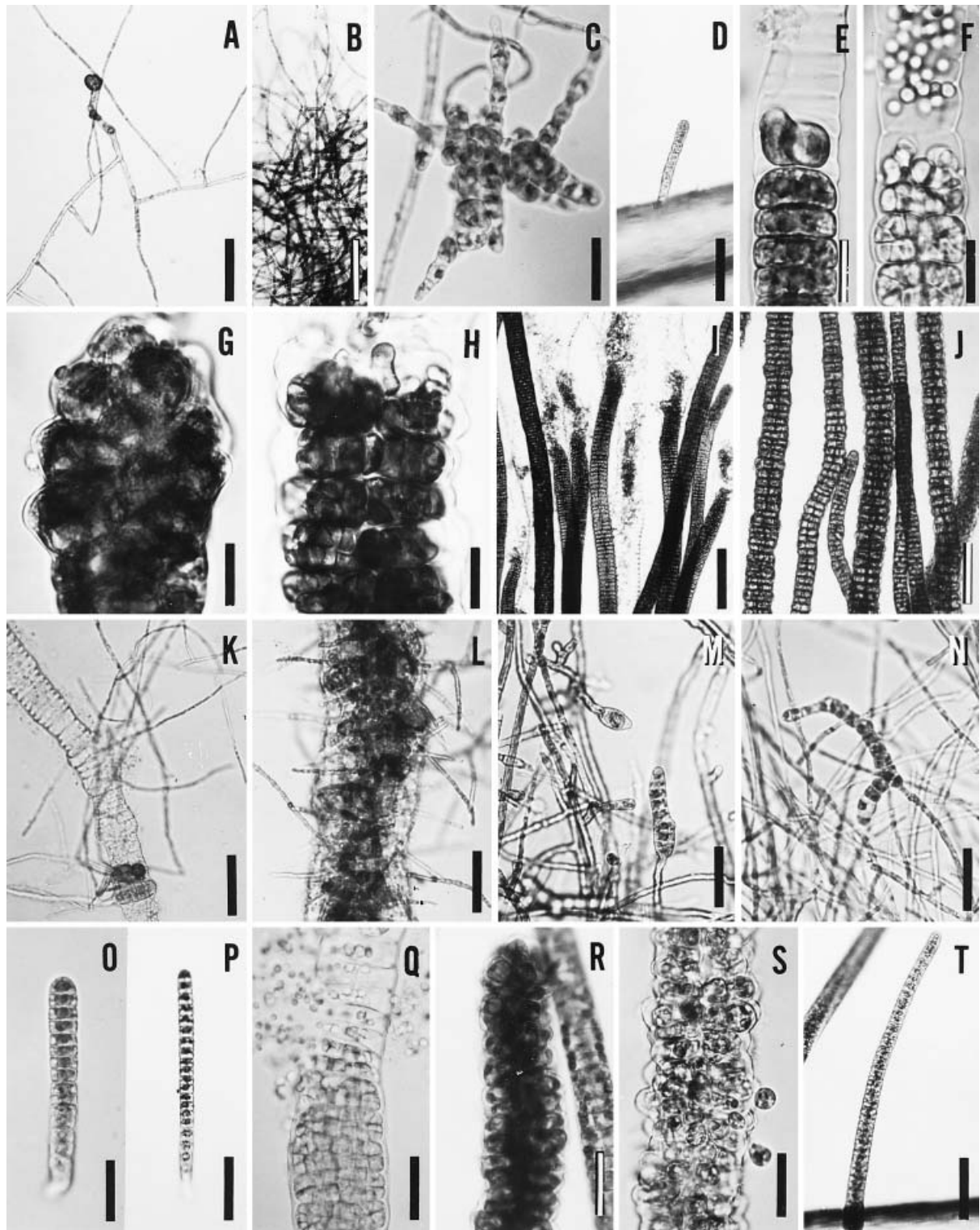
MATERIALS AND METHODS

Plant materials of *B. atropurpurea* (Roth) Lyngbye were collected on 30 November 1991 at Fukaura, Aomori Prefecture and on 3 March 1998 at Enoshima, Kanagawa Prefecture, Japan. To obtain zygotospores (following the terminology of Guiry¹⁰) or archeospores (following the terminology of Magne¹¹) in culture, fragments of plants were cut off from the upper portion of mature

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plants. The surface of fragments was cleaned with an artist brush in sterilized seawater, and kept overnight in antibiotic solution.¹² On the following day, these fragments were placed in Petri dishes containing sterilized seawater for several hours to obtain archeospores or zygospores. After a week in culture, zygospore and archeospore germlings of the Fukaura plant and archeospore germlings of the Enoshima plant were isolated by a glass pipette and transferred into a new Petri dish with sterilized seawater to establish unialgal culture. Conchocelis filaments and conchospores were cultured in glass tubes containing 50 mL medium at 5, 10, 15, 20, 25 and 30°C, under a photon flux density of 80 $\mu\text{mol}/\text{m}^2/\text{s}$ and day lengths of 14 L : 10 D and 10 L : 14 D.

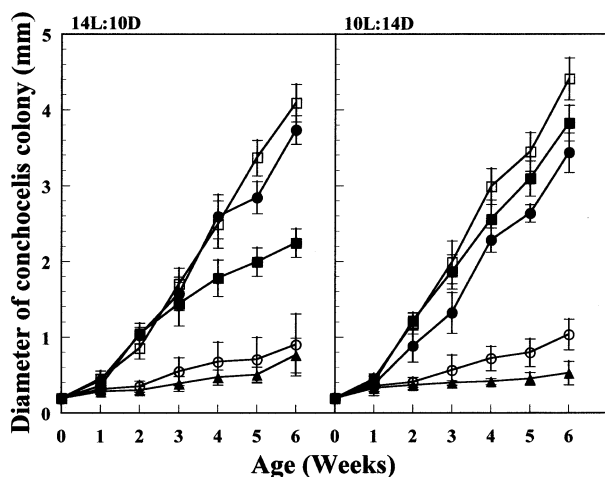


Fig. 2 Influence of temperature and day length under a light intensity of 80 $\mu\text{mol}/\text{m}^2/\text{s}$ on the growth (\pm SD) of the conchocelis colony of *Bangia atropurpurea* from Fukaura, Aomori Prefecture. ■, 30°C; □, 25°C; ●, 20°C; ○, 15°C; ▲, 10°C.

Over 100 conchospores were isolated into each Petri dish by capillary pipettes and cultured for 6 weeks at 20°C and 80 $\mu\text{mol}/\text{m}^2/\text{s}$ under 14 L : 10 D. After 6 weeks, the sexuality of the germlings was examined. Over 100 archeospores from the male or female gametophyte were isolated and cultured at 20°C and 80 $\mu\text{mol}/\text{m}^2/\text{s}$ under 14 L : 10 D. After 6 weeks, the sexuality of the archeospore germlings was examined.

Apogamic filaments produced from the male and female gametophytes of the Fukaura plant were cultured at 20°C and 80 $\mu\text{mol}/\text{m}^2/\text{s}$ under 14 L : 10 D. Conchospores from the apogamic filaments were cultured under the same conditions as above. Sexuality of the conchospore germlings was examined.

A modified Grund medium¹³ was used in the culture, the medium was renewed every 4 days.

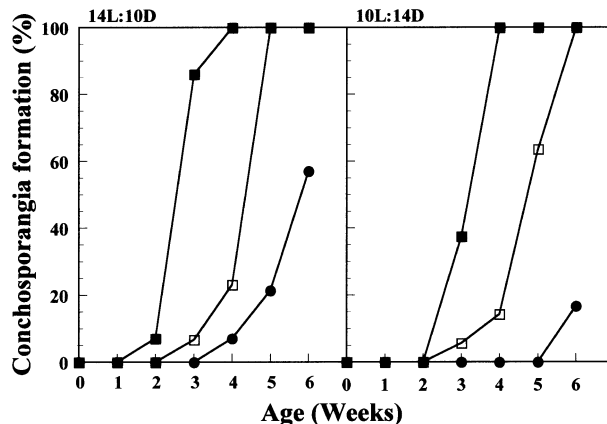


Fig. 3 Influence of temperature and day length under a light intensity of 80 $\mu\text{mol}/\text{m}^2/\text{s}$ on the formation (%) of a conchosporangial branch of *Bangia atropurpurea* from Fukaura, Aomori Prefecture. ■, 30°C; □, 25°C; ●, 20°C.

Fig. 1 Life histories of *Bangia atropurpurea* from Fukaura, Aomori Prefecture (A–R) and from Enoshima, Kanagawa Prefecture (S–T) at 20°C and 80 $\mu\text{mol}/\text{m}^2/\text{s}$ under 14 L : 10 D in culture. (A) Zygospore germling after 2 weeks. (B) Conchocelis colony after 3 weeks. (C) Conchosporangial branches after 5 weeks. (D) Conchospore germling after 4 days. (E) Liberating archeospores from the tip of a young gametophyte after 12 days. (F) Matured male gametophyte after 20 days. (G) Mature female gametophyte after 32 days. (H) Liberating zygospores at the tip of a female gametophyte after 40 days. (I) Archeospore germlings from male gametophytes produced spermatangium after 40 days. (J) Archeospore germlings from female gametophytes produced carpogonium after 40 days. (K) Apogamic conchocelis produced directly from the cells of a male gametophyte after 6 weeks in culture. (L) Apogamic conchocelis produced directly from the cells of a female gametophyte after 6 weeks in culture. (M) Conchosporangial branches were produced on the apogamic filaments from the male gametophyte. (N) Conchosporangial branches were produced on the apogamic filaments from the female gametophyte. (O) Conchospore germling from the apogamic filaments from the male gametophyte. (P) Conchospore germling from the apogamic filaments from the female gametophyte. (Q) Mature male gametophyte of the conchospore germlings from the apogamic filaments from the male gametophyte. (R) Mature female gametophytes of the conchospore germlings from the apogamic filaments from female gametophyte. (S) Liberating archeospores from the Enoshima plant after 40 days. (T) Archeospore germlings of Enoshima plant after 4 days. Scale bars: 200 μm in (B) and (I,J); 80 μm in (A) (D) (K–N) (P) and (T); 40 μm in (C) (O) and (Q–S); 20 μm in (E–H).

RESULTS

Life history of the plant from Fukaura and Enoshima

Morphological characteristics of the wild plant of *B. atropurpurea* from Fukaura and Enoshima were not different regarding the length (47.4 ± 3.5 mm and 47.2 ± 3.7 mm) and diameter (98.7 ± 10.7 μ m and 123 ± 23 μ m) without the sexual reproductive cells. Life history alternating conchocelis and gametophyte was observed in the Fukaura plant. Archeospore liberation occurred from the male and

female gametophytes of the Fukaura plant. However, sexual reproductive cells were not observed in the Enoshima plants (ca. 500 individuals).

Life history of both plants in culture was observed at 20°C and 80 μ mol/m²/s under 14 L:10 D. Zygotospores from the wild female gametophytes of Fukaura plants germinated into conchocelis (Fig. 1A). The conchocelis colonies grew to approximately 1.5 mm diameter and produced conchosporangial branches after 4 weeks in culture (Fig. 1B,C). Liberated conchospores attached to the substrate and germinated into the gametophytes (Fig. 1D). Archeospores were liberated from the tip of the gametophyte at approximately 0.2 mm length of plant after 12 days in

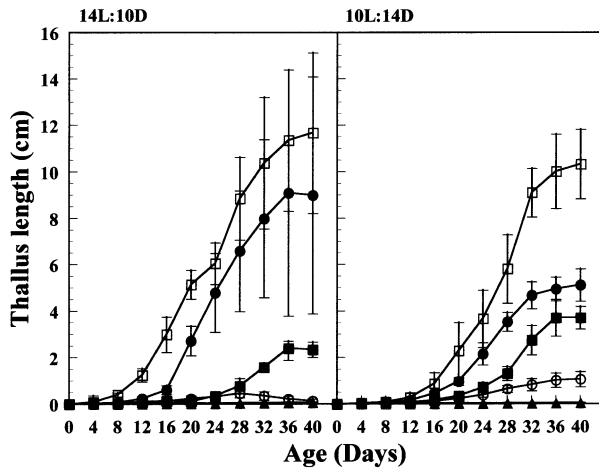


Fig. 4 Influence of temperature and day length under a light intensity of 80 μ mol/m²/s on the growth (\pm SD) of gametophyte of *Bangia atropurpurea* from Fukaura, Aomori Prefecture. ■, 30°C; □, 25°C; ●, 20°C; ○, 15°C; ▲, 10°C.

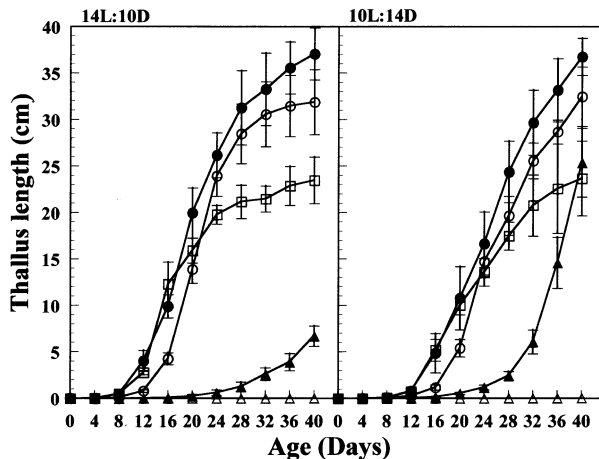


Fig. 5 Influence of temperature and day length under a light intensity of 80 μ mol/m²/s on the growth (\pm SD) of *Bangia atropurpurea* from Enoshima, Kanagawa Prefecture. □, 25°C; ●, 20°C; ○, 15°C; ▲, 10°C; △, 5°C.

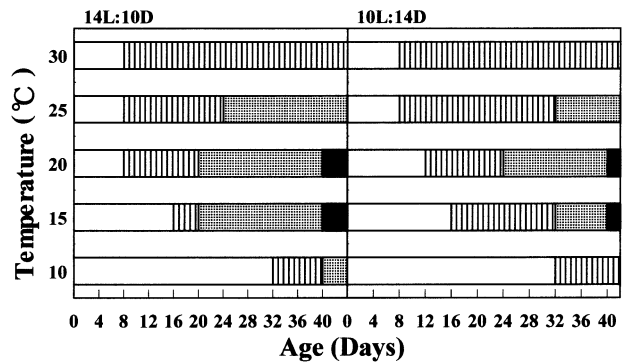


Fig. 6 Liberation of archeospores, spermata and zygotospores in relation to the culture age of the gametophyte of *Bangia atropurpurea* from Fukaura, Aomori Prefecture at different temperatures and day length under a light intensity of 80 μ mol/m²/s. □, Neither archeospores, spermata nor zygotospores liberated; ▤, archeospores liberated; ▨, archeospores and spermata liberated; ■, archeospores, spermata and zygotospores liberated.

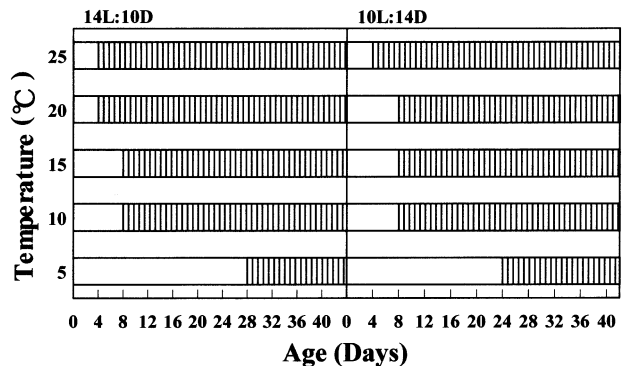


Fig. 7 Liberation of archeospores in relation to the culture age of *Bangia atropurpurea* from Enoshima, Kanagawa Prefecture at different temperatures and day lengths under a light intensity of 80 μ mol/m²/s. □, No archeospores liberated; ▤, archeospores liberated.

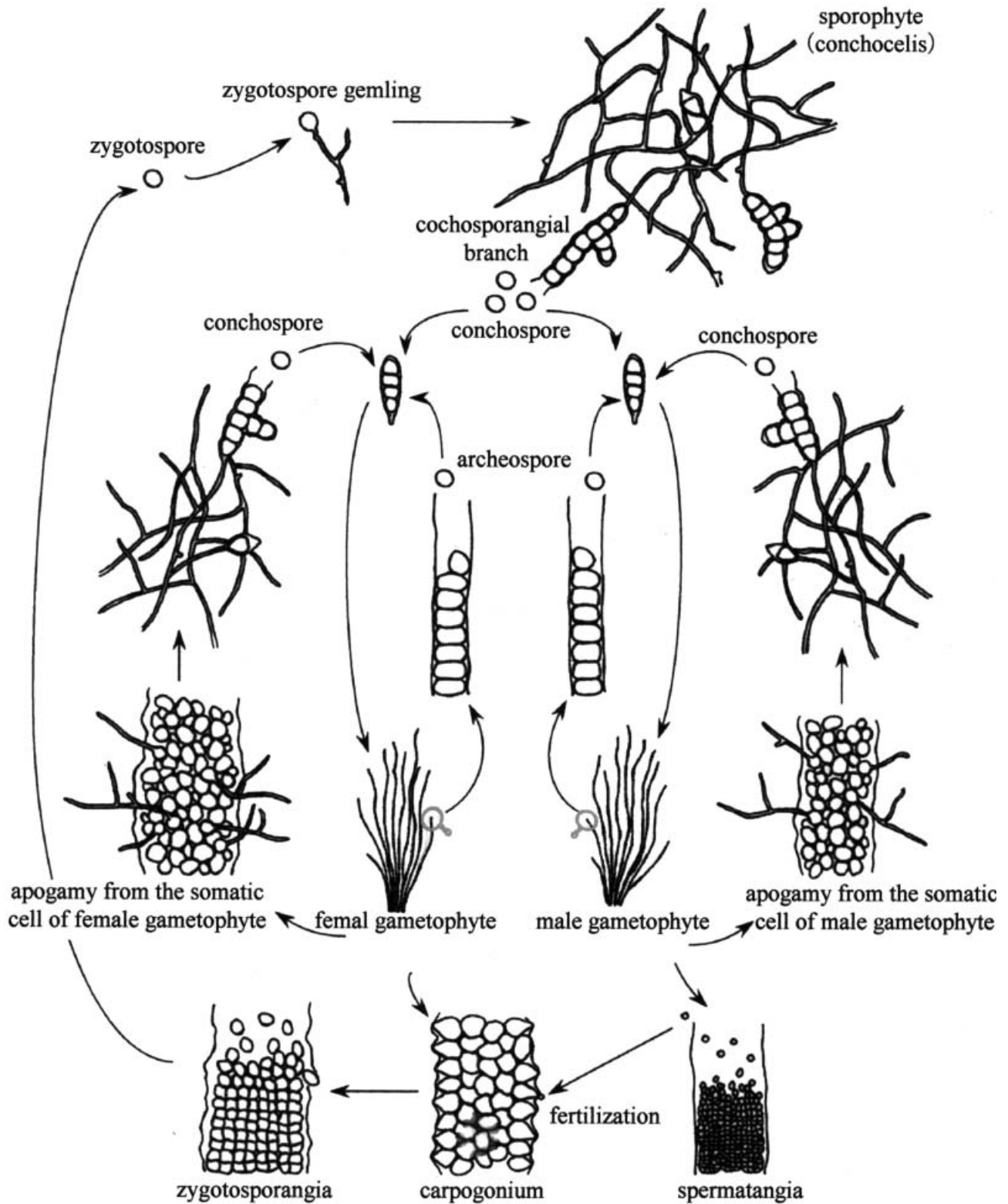


Fig. 8 Summarized life history of *Bangia atropurpurea* from Fukaura, Japan.

culture (Fig. 1E). After 20 days in culture, the cells at the upper part of the male gametophytes became pale yellow in color and were divided into smaller cells, and produced spermatia (ca. 5 μm diameter) (Fig. 1F). The female gametophytes produced carpogonia after 32 days in culture, and liberated spermatia were observed on the cell wall (Fig. 1G). After 40 days in culture, zygotospores (ca. 20 μm diameter) were liberated from the female gametophytes (Fig. 1H). In cases of the absence of the male gametophyte in the same Petri dish, the isolated female gametophyte produced carpogonia, however, they did not produce zygotospores.

Sexuality of the isolated conchospore germlings of 100 individuals was examined after 6 weeks in culture. Forty-eight individuals as male gametophytes and 52 individuals as female gametophytes were recognized. Sexuality of the archeospore germlings from the isolated male and female gametophytes was examined after 6 weeks in culture. The archeospore germlings from the male and female gametophytes grew into male and female gametophytes, respectively (Fig. 1I,J). Archeospore germlings showed the same sexuality as the mother plant without exception in approximately 100 germlings from each isolated gametophyte.

Apogamic conchocelis was produced directly from the cells of the male and female gametophytes after 6 weeks in culture (Fig. 1K,L). Growth rate of the conchocelis was observed to be similar to the zygotospore germlings. They grew to form colonies and produced conchosporangial branches (Fig. 1M,N). The size and appearance of the filaments and conchosporangial branches were not different from the normal conchocelis (ca. 5 μm diameter) of the zygotospore germlings. Both conchospores germinated into gametophytes (Fig. 1O,P). Sexuality of the gametophyte was the same as the mother plant (Fig. 1Q,R).

Enoshima plants produced archeospores only (Fig. 1S). The size of the archeospore of the Fukaura plants ($19.2 \pm 2.1 \mu\text{m}$ in diameter) was slightly larger than the Enoshima plants ($16.9 \pm 1.8 \mu\text{m}$ in diameter), but the color of the spores was not different in the two plants. The archeospore germlings grew into the asexual plants (Fig. 1T). Male or female plants and filamentous phases as apogamy or conchocelis were not found even after several culture experiments.

Influence of temperature and day length on growth and maturation of the plant and conchocelis

Fast growth of the conchocelis colony of Fukaura plants was observed at 20–30°C under short day

photoperiod (Fig. 2). Growth of the conchocelis colony was very slow at 10–15°C, and did not mature within 6 weeks in culture. At 5°C, the conchocelis died. The formation of conchosporangial branches occurred at 20–30°C (Fig. 3). Early formation of the conchosporangial branches was observed at higher temperatures and under a long day photoperiod.

The influence of temperature and day length on the growth of Fukaura gametophytes and Enoshima plant is shown in Figs 4 and 5.

Optimum temperature for the growth of Fukaura gametophytes and the Enoshima plant was observed at 25°C and 20°C, respectively. Fast growth occurred under long day conditions for both plants. The maximum size of plants within the culture period was clearly different between Fukaura and Enoshima. The size of Fukaura gametophytes was smaller than those from Enoshima under the same culture conditions of 10–25°C (Fig. 4). Both gametophytes did not grow well or died at 5°C and 30°C. Enoshima plants did not grow more than 16 days in culture at 30°C. Both Fukaura and Enoshima plants produced archeospores at 5–25°C and at 10–30°C, respectively. Fukaura plants grew well at 20°C and 25°C, and produced zygotospores at 15–20°C within a culture period of 40 days (Figs 6,7).

DISCUSSION

Some parts of the life history of *Bangia fuscopurpurea* or *B. atropurpurea* have been previously reported and the complete life history was determined for European or North American species.^{3,14–20} Although the life history of Japanese species has been reported previously, there have been no reports on the sexuality of archeospore germlings and apogamy from the vegetative cells of male or female gametophytes.^{9,21,22}

The life history of the Fukaura plant is summarized in Fig. 8. It follows basically a '*Porphyra dentata*-type' life history.²³ However, it has two kinds of filamentous and conchocelis phases (i.e. including a conchocelis phase from the zygotospores germlings and reproduction by apogamy from the vegetative cells of male or female gametophytes). The sexuality of the conchospore germlings produced from the conchosporangia of the apogamic conchocelis is the same as the sexuality of the mother plant. Therefore, it may be haploid on the apogamic conchocelis, and meiosis and sex determination may not have occurred in the apogamic conchocelis. In future, we need to find out the position of the meiosis or sex determination.

From the results of over 10 culture experiments and from more than 100 plants under several experimental conditions, Fukaura and Enoshima plants always demonstrated a sexual-biphasic cycle and an asexual cycle by archeospores only, respectively. It seems that the life history exhibited is similar to freshwater plants of *B. atropurpurea*.²⁻⁶ The life history type of marine species of *Bangia* is reported from Japan for the first time.

Asexual and sexual plants from Enoshima and Fukaura showed remarkably different responses in growth rate and the maximum plant length in culture. Enoshima plants grew faster and became three to fourfold bigger than the Fukaura plants at 20°C.

From these observations of the different life histories and physiological responses, it may be suggested that sexual and asexual marine plants of *Bangia* occur in two localities. In the future, we need to undertake more detailed field and molecular observations on the two populations.

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