Journal of Applied Phycology (2006) 18: 279–286
DOI: 10.1007/s10811-006-9027-9

Developmental studies in *Porphyra vietnamensis*: A high-temperature resistant species from the Indian Coast

Dinabandhu Sahoo*, Pooja Baweja & Neetu Kushwah
Marine Biotechnology Laboratory, Department Of Botany, University of Delhi, Delhi – 110007, India

*Author for correspondence: e-mail: dbsahoo@hotmail.com

Key words: *Porphyra*, development, high temperature, India

Abstract

Porphyra vietnamensis Tanaka & Pham-Hoang Ho (Bangiales, Rhodophyta) is a tropical seaweed collected from the west coast of India. Thalli of the blade phase are found growing only during the rainy season between July and September. They grow on rocky intertidal or subtidal substrata or as epiphytes on other seaweeds such as *Enteromorpha flexuosa* and *Chaetomorpha media*. The gametophytic thallus is monostromatic and covered with spines at the base. The species is monoecious. Male gametangia are found in patches that are distributed in the upper part of the thallus. Archeospores are found at the thallus margins and give rise to the blade phase after one week of germination even at 30 °C. Zygotospores germinated at 25 °C into conchocelis within three days from the date of their inoculation. Conchospores were released at 30 °C. The young blades grew at 32 °C in the laboratory.

Introduction

Porphyra (Bangiales, Rhodophyta) is one of the world's most valued maricultured seaweeds, and is primarily used as food in many oriental countries. It is highly prized for its flavour and as a health food as it is rich in proteins and vitamins. Nearly 17 types of free amino acids, including taurine, which controls blood cholesterol levels (Tsujii et al., 1983) can be found within the genus, which has an annual value of over US$ 1.8 billion (Yarish et al., 1999). The biology and ecology of *Porphyra* has been studied more thoroughly than that of any other red algal genus (Tseng & Sun, 1989; Cole, 1990; Hawkes, 1990). Recently, it has been reported that *Porphyra* has much more potential and can be used as an experimental system for modern biological research, like *Arabidopsis thaliana* (Sahoo et al., 2002). *Porphyra* is represented by more than 133 species, which are particularly abundant in cold-temperate and boreal shores of the Northern and Southern Hemispheres (Yoshida et al., 1997). In some areas, individual species grow throughout the year whereas other species are very seasonal, with big crops present on the rocks for only one to two months of the year. So far seven species of *Porphyra*, namely *P. chauhanii* Anil Kumar & Panikkar, *P. indica* V. Krishnamurthy & Baluswami, *P. kanyakumariensis* V. Krishnamurthy & Baluswami, *P. crispata* Kjellman, *P. okhaensis* H. Joshi, Oza & Tewari, *P. suborbiculata* Kjellman, and *P. vietnamensis* T. Tanaka & Pham-Hoang Ho have been reported from different parts of the Indian coast, of which *P. vietnamensis* is the most abundant (Sahoo et al., 2001). Børgesen (1937) reported *P. vietnamensis* as *P. tenera* Kjellman from Madras Harbour. Later, Sreeramulu (1952) originally described the plants as *P. naidum* Anderson. Subsequently, Umamaheswara Rao and Sreeramulu (1963) confirmed the species as *P. vietnamensis*. Lewmanomont and Ogawa (1978) studied the life cycle of *P. vietnamensis* from Songkhla, Thailand but were not successful in obtaining the release of conchospores from the conchocelis phase. Imada and Abe (1980) were able to get conchospores from *P. vietnamensis* by using phytohormones. Lewmanomont and Chittpoolkusol (1993) studied the life history of *P. vietnamensis* from Thailand and were able to complete the life history in the

laboratory at 25 °C. Although *P. vietnamensis* has been reported from different parts of the Indian coast, no detailed studies have been undertaken on its development in culture. In the present study, it has been found that a particular strain of *P. vietnamensis* can complete its life history at 32 °C in the laboratory. Since most of the *Porphyra* species grow at lower temperatures, this Indian strain of *P. vietnamensis* could be a potential crop for mass cultivation in tropical seas.

Materials and methods

Porphyra vietnamensis was collected during July 2003 from the rocky coast of Goa in western India. Fertile blades were selected and cleaned with seawater in the field to remove visible epiphytes and other contaminants. The thalli were wrapped in absorbent cotton moistened with seawater, packed in plastic bags and transported to the laboratory in an air-conditioned railway compartment. In the laboratory, the thalli were washed thoroughly in autoclaved seawater and each thallus was observed under a stereo binocular microscope. Thallus surfaces were cleaned with the help of sterilized cheese cloth and epiphytes were removed. Subsequently, the thalli were washed 3–4 times in autoclaved seawater. Then each thallus was blotted with tissue paper. Individual thalli were then wrapped in cheese cloth, put in a polythene bag and kept in a refrigerator. After two hours thalli were taken out and individual thalli were put into petri-dishes (size 90 mm, Polylab India) separately in three different culture media: f/2 (Guillard & Rhyther, 1962), PES medium (Provasoli, 1968) and autoclaved seawater. The petri-dishes were kept at 20 °C, 25 °C, 30 °C and room temperature (32 °C) under cool white 40 W fluorescent lights at an approximate photon fluence rate of 40 μmol photons m^{-2} s^{-1}. The thalli in petri-dishes were observed after every two hours for the release of spores which were collected by means of Pasteur pipette and transferred to new petri-dishes separately in the above mentioned media. A drop of GeO_2 was added to each petri-dish to prevent the growth of diatoms (1 g GeO_2 dissolved in 100 mL of distilled water). All petri-dishes were kept in the above mentioned temperature and light conditions with a photoperiod of 12:12 h light: dark cycle. The salinity of media was maintained at 25 ppt. Culture media were changed after every seven days and observations on spore development were recorded. Photographs were taken through a Nikon E600 photomicroscope (Nikon, Tokoyo, Japan) using ILFORD Black and White film. Spore terminology follows Nelson et al., 1999.

Observations

Porphyra vietnamensis was found growing predominantly on the west coast of India especially in the Provinces of Maharashtra, Goa and Karnataka. The species was strictly seasonal. The leafy thalli appeared on the rocky substratum, oyster shells (*Crassostrea gryphoides*) or as an epiphyte on seaweeds such as *Enteromorpha flexuosa* (Wulfen) J. Agardh and *Chaetomorpha antennina* (Bory de Saint-Vincent) Kützing in the mid-tidal to spray zone from the beginning of July. The blades started degenerating from the end of August and completely disappeared by the end of September. Over the last several years the senior author has observed that the growth of *Porphyra* species in India is associated with the onset of the monsoon season. During this season the seawater is turbid and rich in nutrients due to river and land-surface discharge. The seawater temperature decreases substantially from 30–32 °C to 19–23 °C and salinity from 31–33 ppt to 19–22 ppt during these months. The sky is mostly cloudy during this season, which appears to favour the growth of the species.

Thallus morphology

Considerable variation was observed in the gross morphology of *P. vietnamensis* but in general the blades were monostromatic, membranous, lanceolate to linear-lanceolate and sometimes ribbon-like, purple to pink-purple in colour. The thalli were attached to the substratum with discoid holdfasts. Sometimes thalli were branched from a common base having several bladelets (Figure 1). Usually the thalli were 3–15 cm in height but sometimes grew up to 40 cm. They were 1.5–3 cm broad and 25–32 μm thick. The cells of the basal regions were large, with pigmented pear-shaped heads, and elongated (Figure 2). Margins were undulate with 2–3 celled spines, which were found towards the basal region (Figure 3). The vegetative cells in the central region were vacuolated with a single stellate plastid (Figure 4). The thalli were monoecious with distinct male gametangial and zygotosporangial patches found towards the margin and these were present on the same thallus. The male gametangia could be distinguished as pale-yellow patches at the outer margins. There were 64 spermatia per male gametangium arranged in 4 tiers

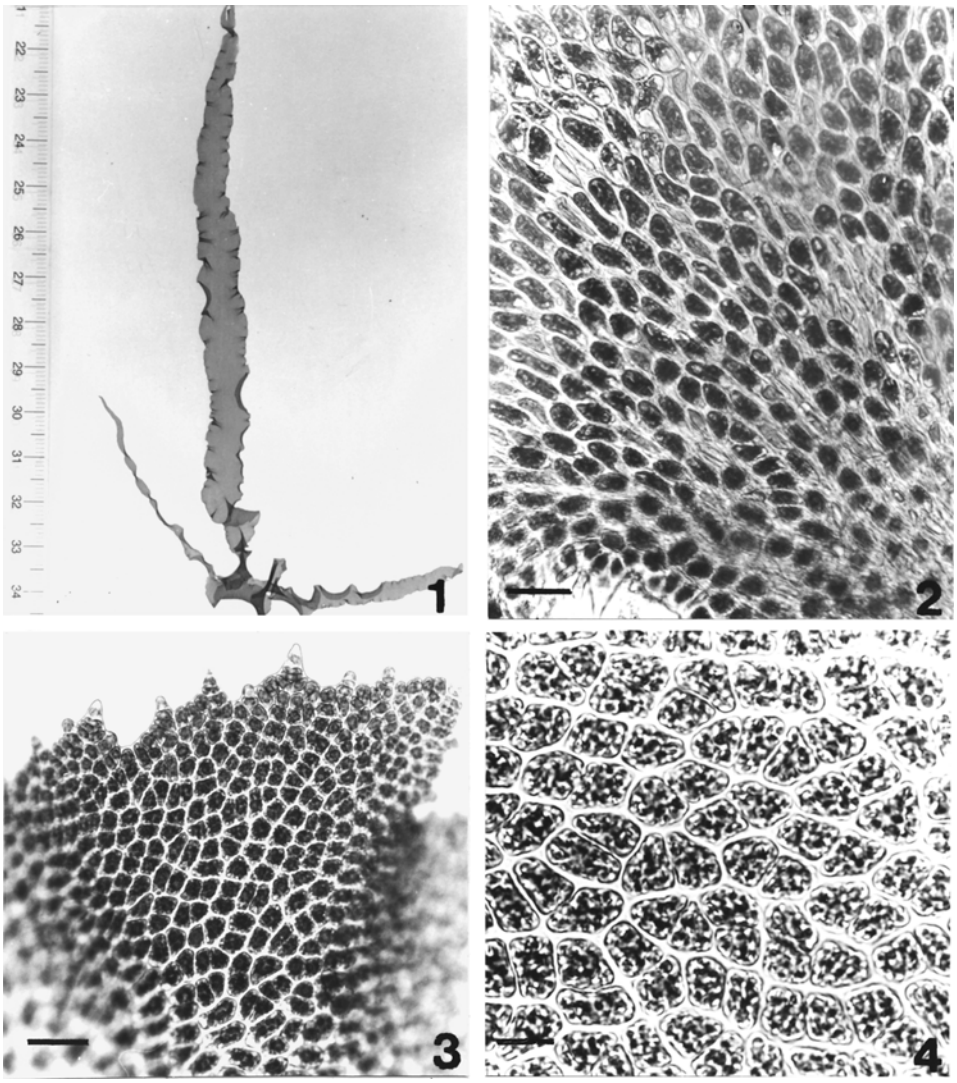


Figure 1–4. Porphyra vietnamensis. Thallus morphology. (1). Mature thallus of *Porphyra vietnamensis* showing branching at the base. (2). Basal portion showing pear shaped cells. Scale bar = 100 μm. (3). Margin showing spines. Scale bar = 100 μm. (4). Surface view of central region showing vegetative cells. Scale bar = 20 μm.

of 16 each, having the spore formula a/4, b/4, c/4. There were 8 zygotospores arranged in two tiers of 4 each, having the spore formula a/2, b/2, c/2.

Developmental studies

In culture the gametophytic thalli produced two types of spores: archeospores which germinated directly to give rise to the young blades, and zygotospores which gave rise to concohocelis. The conchocelis produced conshocporangia which released conchospores that developed into blades.

Archeospore development

Archeospores were released from the margins of the mature blades in all the temperature and light conditions tested. The spores were round, larger in size than zygotospores, vacuolated, thick walled and were between 18–20 μm in diameter (Figure 5). The spores germinated unipolarly after 24 h of release. After 48 h, the spores divided to form a 2-celled structure where one of the cells showed distinct polarity (Figure 6). The spores divided and formed young germlings (minute blades) in between 4–6 days (Figures 7 and

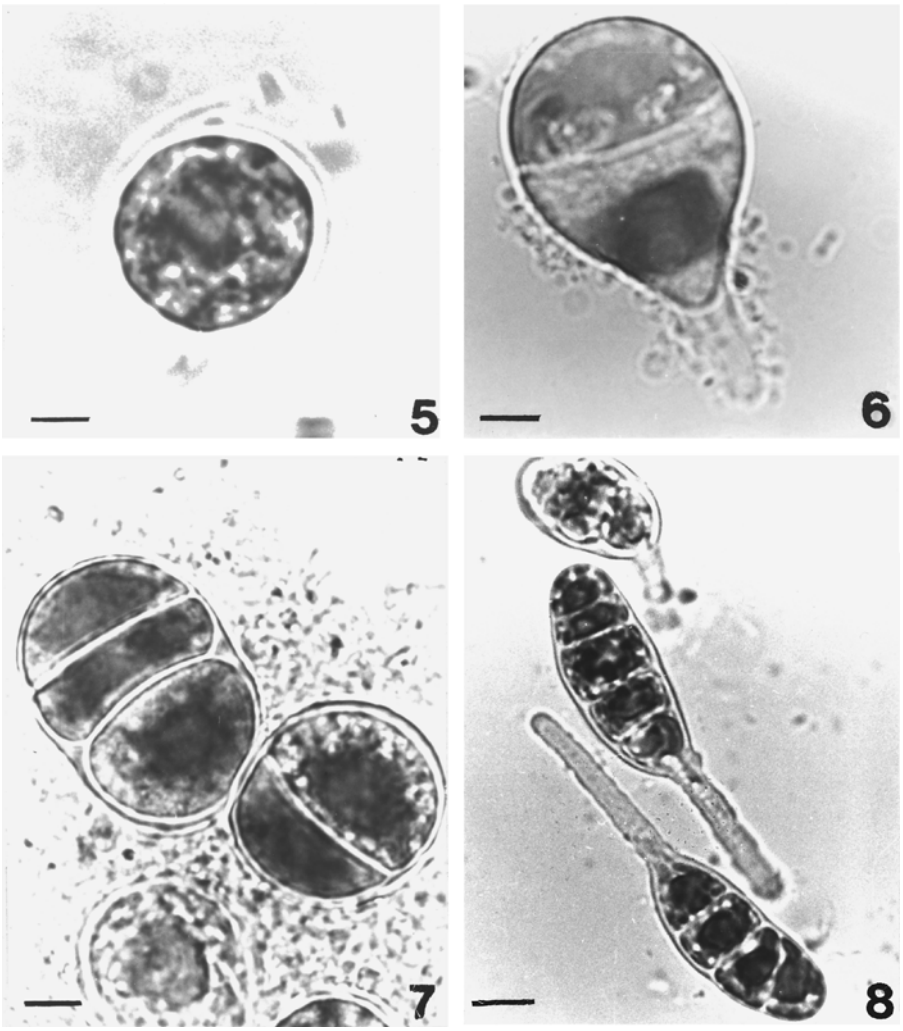
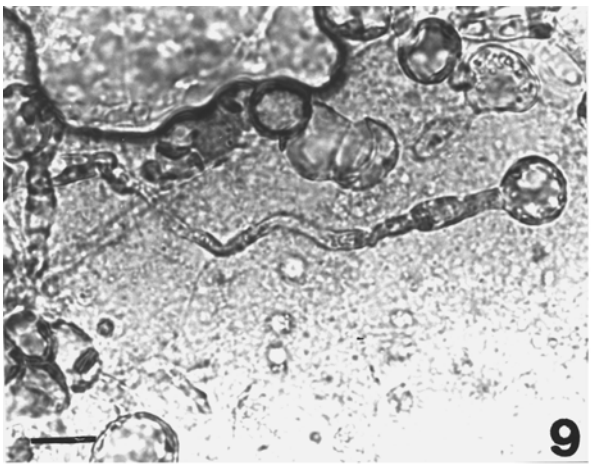


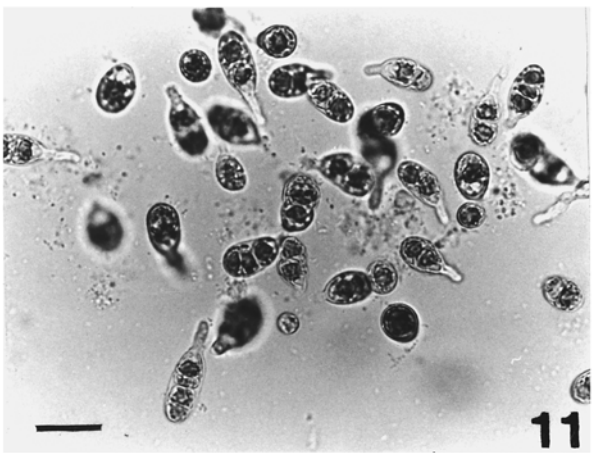
Figure 5–8. *Porphyra vietnamensis*: archeospore development. (5). A thick walled archeospore. Scale bar = 10 μm. (6). Two celled stage showing polarity. Scale bar = 10 μm. (7). 1–3 celled stages. Scale bar = 20 μm. (8). Young germlings. Scale bar = 20 μm.

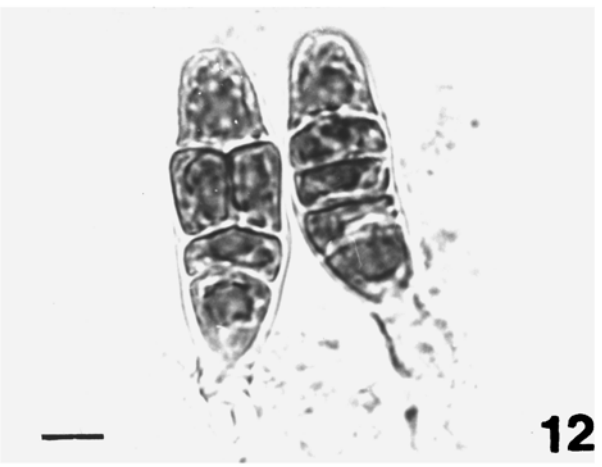
8), completing the asexual mode of life history in less than one week. When cultured for a longer period, i.e. 45–60 days, the thalli developed normal morphology (i.e. similar to field material) at 20, 25 and 30 °C (Figure 13) but at 32 °C showed abnormal thallus morphology (Figure 14).

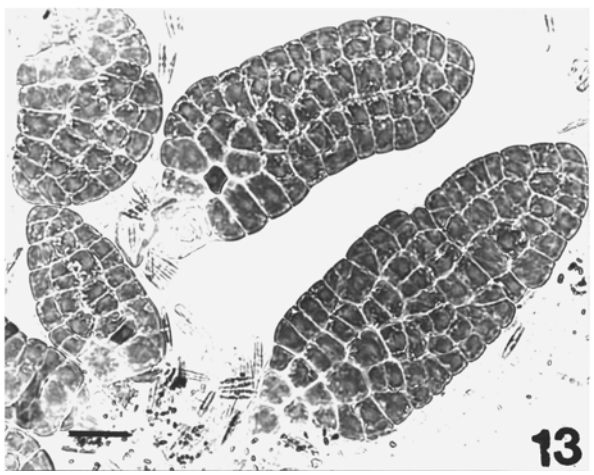
Conchocelis development

Zygotospores were released from the margins of the fertile blade in 24–48 h in all the above temperature and light conditions mentioned earlier. The spores were thick-walled, and between 12–15 μm in diameter, pinkish in colour and each with a distinct, stellate plastid. The zygotospores germinated unipolarly between 48–72 h after release (Figure 9). Subsequently, the spores developed into conchocelis filaments of indeterminate growth with extensive branching (Figure 10). The growth of conchocelis was slower at 20 °C whereas it grew rapidly at 25 and 30 °C but could still survive and remain healthy at 32 °C. When the conchocelis was allowed to grow in the petri-dish, it formed an extensive network of filaments which were attached to the bottom of the dish, whereas it formed spherical colonies or balls when grown in a conical flask. The conchocelis filaments were light pink in colour.









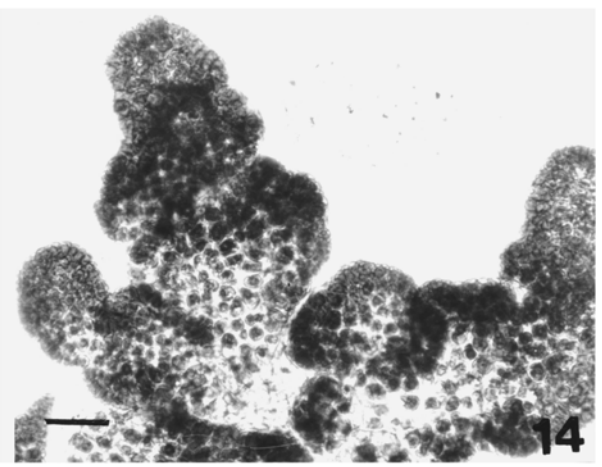


Figure 9–14. *Porphyra vietnamensis*: development of zygotospores. (9). Zygotospores showing unipolar germination into conchocelis. Scale bar = 50 μm. (10). Free living conchocelis growing at room temperature (32 °C). Scale bar = 50 μm. (11). Conchospores and young germlings in different stages of development at 20 °C–25 °C. Scale bar = 20 μm. (12). Young sporelings showing different planes of cell divisions. Scale bar = 20 μm. (13). Young thalli grown at 25 °C. Scale bar = 50 μm. (14). Formation of abnormal thalli at room temperature (32 °C). Scale bar = 10 μm.

Formation of conchosporangia and conchospore release

At 20 °C, 25 °C and 30 °C, conchosporangia were formed on the conchocelis filaments after one month of release of zygotospores. However, the best growth of conchosporangia was observed at 30 °C in day-neutral conditions at a light intensity of 40 μmol photons $m^{-2}s^{-1}$. Conchospores were released both at 25 and 30 °C. Conchospores germinated bipolarly which gives rise to small, uniseriate filaments whose basal cells germinated into rhizoids (Figure 11). The middle and apical cells of the filaments divided longitudinally and transversely, forming young gametophytic thalli (Figure 12).

Germling development in culture

The blades grew well and showed normal morphology (similar to the field material) at 25 and 30 °C in the laboratory (Figure 13). The reproductive structures were observed after 30 days of growth. However, at room temperature (32 °C) the thalli showed abnormal morphology (Figure 14). No reproductive structures were observed in the abnormal thalli, even after 2–3 months of culture.

Discussion

In general, species of *Porphyra* occur in cool to warm temperate waters throughout the world (Holmes & Brodie, 2004). There are several species of *Porphyra* which are perennial such as *P. dioica* Brodie & L. Irvine (Brodie & Irvine, 2003) whereas some species are strictly seasonal. Santelices and Avila (1986) reported that the occurrence of *P. columbina* Montagne is strictly seasonal in central Chile, where the leafy thallus appears in late winter and grows up to midsummer only. Tanaka and Ho (1962) reported the occurrence of *P. vietnamensis* from the Northwest Pacific region of Vietnam. Ogawa and Lewmanomont (1979) reported growth of *Porphyra vietnamensis* only during the rainy season between November and February in Thailand. They have also found this species in Myanmar (Burma) and Malaysia. Lewmanomont and Chittpoolkusol (1993) also reported the occurrence of this species from Songkhla provinces, Thailand during the rainy season when the seawater temperature is 24–27 °C and salinity between 8–26 ppt. Tseng (1983) and Wu (1988) reported the occurrence of the same species from China. In the present study, it has been observed that *Porphyra vietnamensis* is strictly seasonal in India, where gametophytic thalli are found only during the rainy season when the water temperature is between 19 and 23 °C, the salinity goes down to between 19–22 ppt and the nutrient levels are higher in the sea than in the summer and winter seasons. Further, it has been observed by the senior author over the years that if the onset of the monsoon is delayed then the appearance of the gametophytic thalli is accordingly delayed. There have been several studies on the effect of temperature on growth and development of *Porphyra* (Gargiulo et al., 1994; Kim 1999; Katz, et al., 2000) but only a few reports on the effect of salinity on growth and maturation of the blade. Lewmanomont and Chittpoolkusol (1993) reported that salinity is a major factor which influences the life history of *P. vietnamensis*. This has been confirmed by Ruangchuay and Notoya (2003) in a laboratory culture study. Since the temperature and salinity of sea water are lower during the monsoon season, these two factors might be responsible for growth of *Porphyra* during monsoon season in India.

P. vietnamensis, like most other species of *Porphyra,* has a heteromorphic life history with alternation of a foliose leafy gametophytic thallus and a filamentous conchocelis sporophytic phase. The pattern of life history of various *Porphyra* species is determined not only by genetic traits but also by the integrated effects of several environmental factors (Katz et al., 2000). It has been reported that temperature, photoperiod and light intensity are three major environmental factors in *Porphyra* for growth of conchocelis, induction of conchosporangia and release of conchospores (Avila et al., 1986; Waaland et al., 1990). We are in agreement with the above observations. In *P. vietnamensis*, zygotospores germinated unipolarly and developed into conchocelis in accordance with observations of others (Kapraun & Luster, 1980; Knight & Nelson, 1999; Brodie & Irvine, 2003). In the present study, the conchocelis phase of this species has not been found growing in nature where the plants were collected. The conchocelis phase of *Porphyra* is cryptic and difficult to find in nature (Mumford, 1980; Conway & Cole, 1977). In India, the coastal water temperature goes up to 30–33 °C during the summer season. The conchocelis grow in shells as well as other hard substrata in this condition, although they are not visible to the naked eye in the field. This is substantiated by the present study where the conchocelis grows at 30 °C and even survives at 32 °C. In some *Porphyra* species, conchosporangial production requires a specific environmental stimulus, such

as short-day conditions, as in *P. tenera* (Dring, 1967). In the present study, conchosporangia were formed in *P. vietnamensis* without the application of any environmental stimuli, as reported in *P. miniata* (Lyngbe) C. Agardh, *P. angusta* Okamura et Ueda and *P. torta* Krishnamurthy (Chen et al., 1970; Chiang & Wang, 1980). Photoperiod and temperature are the most important factors regulating the formation and release of conchospores in the conchocelis phase of *Porphyra* spp. (Edwards, 1969; Freshwater & Kapraun, 1986; Waaland et al., 1987, 1990; Garguilo et al., 1994). However, in *P. vietnamensis*, the conchospores were released without any change in temperature and photoperiod. Generally, in temperate species the optimum growth temperatures of blade and conchocelis are different. For example, *P. lacerata* Miura (Notoya & Nagaura, 1998), *P. moriensis* Ohmi (Notoya & Miyashita, 1999), *P. pseudolinearis* Ueda and *P. dentata* Kjellman (Kim, 1999) have lower temperature (5–15 °C) requirements for the blades and higher temperature (20 °C) for the conchocelis phase. The present study confirms that *P. vietnamensis* does not require such a wide range of temperature induction. Both the blade and conchocelis phases can be grown at 25–30 °C.

The archeospores germinated directly to give rise to young blades, thus completing the asexual life history within a week. It has been observed that the blades of *P. vietnamensis* can grow well even at a high temperature, i.e. at 32 °C, which is quite uncommon in *Porphyra.* From these results we conclude that *P. vietnamensis* from India is a potential species for large-scale mariculture in tropical water.

References

Avila M, Santelices B, McLachlan J (1986) Photoperiodic and temperature regulation of the life history of *Porphyra columbina* (Rhodophyta, Bangiales) from central Chile. Can. J. Bot. 50: 1867–1872.

Børgesen F (1937) Contribution to a South Indian marine algal flora – II. J. Ind. Bot. Soc. 16: 1–56.

Brodie JA, Irvine LM (2003) Seaweeds of the British Isles. Vol. 1. Rhodophyta. Part 3B. Bangiophycidae. Intercept, Hampshire, U.K. 167 pp.

Chen LC-M, Edelstein T, Ogata E, McLachlan J (1970) The life history of *Porphyra minata*. Can. J. Bot. 48: 385–389.

Chiang Y-M, Wang J-C (1980). A study on the production of conchosporangia in the conchocelis phase of *Porphyra angusta* Okamura et Ueda. Phycologia 19: 20–24.

Cole KM (1990) Chromosomes. In Cole, KM, Sheath, RG (eds.), Biology of the red algae. Cambridge University Press, New York: 73–101.

Conway E, Cole K (1977) Studies in the Bangiaceae: structure and reproduction of the conchocelis of *Porphyra* (Bangiales, Rhodophyceae). Phycologia 16: 205–216.

Dring MJ (1967) Effects of daylength on growth and reproduction of the conchocelis-phase of *Porphyra tenera*. J. Mar. Biol. Ass. U.K. 47: 501–510.

Edwards P (1969) Field and culture studies on the seasonal periodicity of growth and reproduction of selected Texas benthic marine algae. Contrib. Mar. Sci. 14: 59–114.

Freshwater WD, Kapraun F (1986) Field, culture and cytological studies on *Porphyra carolinensis* Coll et Cox (Bangiales, Rhodophyta) from North Carolina. Jpn. J. Phycol. 34: 251–262.

Gargiulo GM, Masi FD, Genovese G, Tripodi G (1994) Karyological and effects of temperature and photoperiod on the life cycle of *Porphyra leucosticta* Thuret in Le Jolis (Bangiales, Rhodophyta) from the Mediterranean Sea. Jpn. J. Phycol. 42: 271–280.

Guillard RRL, Rhyther JH (1962) Studies of marine planktonic diatoms. I. *Cyclotylla nana* Hustedt and *Detumula confervacea*. Can. J. Microbiol. 8: 229–239.

Hawkes MW (1990) Reproductive strategies. In Cole, KM, Sheath, RG (eds.), Biology of the Red Algae. Cambridge University Press, New York: 455–476.

Holmes MJ, Brodie J (2004). Morphology, seasonal phenology and observations on some aspects of the life history in culture of *Porphyra dioica* (Bangiales, Rhodophyta) from Devon, U.K. Phycologia 43: 176–188.

Imada O, Abe T (1980) Formation and germination of conchospores in *Porphyra vietnamensis*. Bull. Jpn. Soc. Sci. Fish. 46(8): 1051.

Kapraun DF, Luster DG (1980). Field and culture studies of *Porphyra rosengurtii* Coll et Cox (Rhodophyta, Bangiales) from North Carolina. Bot. Mar. 33: 449–457.

Katz S, Kizner Z, Dubinsky Z, Friedlander M (2000) Responses of *Porphyra linearis* (Rhodophyta) to environmental factors under controlled culture conditions. J. Appl. Phycol. 12: 535–542.

Kim N-G (1999) Culture studies of *Porphyra dentata* and *Porphyra pseudolinearis* (Bangiales, Rhodophyta), two dioecious species from Korea. Hydrobiologia 398/399: 127–135.

Knight G, Nelson WA (1999) An evaluation of characters obtained from life history studies for distinguishing New Zealand *Porphyra* species. J. Appl. Phycol. 11: 411–419.

Lewmanomont K, Ogawa H (1978) Study of the life cycle of *Porphyra* of Thailand. A report of the Faculty of Fisheries, Kasetsart University, Bangkok.

Lewmanomont K, Chittpoolkusol O (1993) Life cycle of *Porphyra vietnamensis* Tanaka and Pham-Hoang Ho from Thailand. Hydrobiologia 260/261: 397–400.

Mumford, TF Jr (1980) The reproductive biology *in vitro* of nine species of *Porphyra* (Bangiales, Rhodophyta) from Washington. J. Phycol. 16 (suppl.): 29.

Nelson, WA, Brodie, J, Guiry, MD (1999) Terminology used to describe reproduction and life history stages in the genus *Porphyra* (Bangiales, Rhodophyta). J. Appl. Phycol. 11: 407–410.

Notoya, M, Miyashita, A (1999) Life history, in culture, of the obligate epiphyte *Porphyra moriensis* (Bangiales, Rhodophyta). Hydrobiologia 398/399: 121–125.

Notoya, M, Nagaura, K (1998) Life history and growth of the epiphytic thallus of *Porphyra lacerate* (Bangiales, Rhodophyta) in culture. Algae 13: 207–211.

Ogawa H, Lewmanomont, K (1979) The *Porphyra* of Thailand – II. Distribution and seasonal occurrence of *Porphyra vietnamensis* Tanaka et P-H. Ho. Jpn. J. Phycol. 27: 95–98.

Provasoli, L (1968) Media and products for the cultivation of marine algae. In Watanabe, A & A Hattori (eds.), Cultures and Collections of Algae. Japanese Society of Plant Physiology, Tokyo.

Ruangchuay, R, Notoya, M (2003) Physiological responses of blade and conchocelis of *Porphyra vietnamensis* Tanaka et Pham-Hoang Ho (Bangiales, Rhodophyta) from Thailand in culture. Algae 18: 21–28.

Sahoo, DB, Nivedita, Debasish (2001) Seaweeds of Indian Coast, APH Publishing Corporation, New Delhi, India, pp. 283.

Sahoo, DB, Tang X, Yarish, C (2002) *Porphyra* – the economic seaweed as a new experimental system. Current Science 83: 1313–1316.

Santelices, B, Avila, M (1986) Bases biologicas para maximizar cosecha de "Luche" (*Porphyra columbina* Montagne) en Chile Central. Actas II congress. Algas Marina Chilenas : 201–211.

Sreeremulu, T (1952) On a *Porphyra* from Waltair Coast. Science and Culture 18: 285–286.

Tanaka, T, Ho, P-H (1962) Notes on some marine algae from Viet-Nam I. Memoirs of the Faculty of Fisheries, Kagoshima University 11: 24–40.

Tseng, CK (1983) Common seaweeds of China, Science Press, Beijing, pp. 316.

Tseng, CK, Sun, A (1989) Studies on the alternation of the nuclear phases of chromosome numbers in the life history of some species of *Porphyra* from China. Bot. Mar. 32: 1–8.

Tsujii, K, Kchikawa, T, Matusuura, Y, Kawamura, M (1983) Hypercholestrolemic effect of taurocyamine or taurine on the cholesterol metabolism in white rats. Sulphur Amino acids 6: 239–248.

Umamaheswara, Rao, M, Sreeramulu, T (1963) Vertical zonation and seasonal variation in the growth of *Porphyra* on Visakhapatnam coast. Current Science 32: 173–174.

Waaland, JR, Dickson, LG, Carrier, JE (1987) Conchocelis growth and photoperiodic control of conchospore release in *Porphyra torta* (Rhodophyta). J. Phycol. 23 : 399–406.

Waaland, JR, Dickson, LG, Duffield, ECS (1990) Conchospore production and seasonal occurrence of some *Porphyra* species (Bangiales, Rhodophyta) in Washington State. Hydrobiologia 204/205: 453–459.

Wu, C (1988) The seaweed resources of China. In Critchley AT, Ohno M (eds.), Seaweed resources of the world. Kanagawa International Fisheries Training Center. Japan International Cooperation Agency : 34–46.

Yarish, C, Chopin, T, Wilkes, R, Mathieson, AC, Fei, XG, Lu, S (1999) Domestication of nori for Northern America: The Asian experience. Bull. Aquacult. Ass. Canada 1: 11–17.

Yoshida, T, Notoya, M, Kikuchi, N, Miyata, M (1997) Catalogue of species of *Porphyra* in the world, with special reference to the type of locality and bibliography. Nat. Hist. Res. Special Issue 3: 5–18.