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Karyology and effects of temperature and photoperiod on the life-cycle of *Porphyra leucosticta* Thuret in Le Jolis (Bangiales, Rhodophyta) from the Mediterranean Sea

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Porphyra from the Strait of Messina (Italy), previously referred to *P. leucosticta* Thuret in Le Jolis, was the subject of field and laboratory studies. Cross-gradient light-temperature culture indicate that conchospore formation requires short daylength (9L : 16D) and a temperature of 15–18°C. Conchospore release requires short daylength and a temperature of 18°C. Results of culture investigations are used to explain the seasonal periodicity of growth and reproduction of this species in the study site. The life history in culture involves an alternation between a macroscopic blade and microscopic filamentous conchocelis phase. Typically, blades develop from conchospores with bipolar development while conchocelis develops from carpospores with unipolar development. In addition, both blade and filamentous phases exhibit direct development. Three chromosomes (n=3) were observed in vegetative blade cells, spermatia and vegetative conchocelis cells.

Key Index Words: Conchocelis—*karyology*—*life-cycle*—*photoperiod*—Porphyra leucosticta—*Mediterranean*—*taxonomy*.

The red algal genus *Porphyra* (Bangiales, Rhodophyta) is represented by more than 75 species (Mumford and Cole 1977). Several of these species, which are particularly abundant on cold-temperate and boreal shores of the Pacific and Atlantic Oceans, are of considerable economic significance (Tseng and Sun 1989). Consequently, it is not surprising that the biology, ecology and karyology of *Porphyra* species have been extensively investigated (for rewiews, see Cole 1990, Hawkes 1990, Tseng and Sun 1989).

For many of these species, the life history typically includes a conspicuous blade phase which alternates with a microscopic filamentous conchocelis phase (Cole and Conway 1980). In sexual species, the conchocelis phase is diploid, with meiosis occurring in the germinating conchospore (Burzycki and Waaland 1987, Ohme and Miura 1988), resulting in a mosaic of genetically distinct cellular lines in the haploid blade (Tseng and Sun 1989). Numerous derived asexual life history variations have been reported for *Porphyra* species as well (Kapraun and Lemus 1987, Hawkes 1990).

Considerable information is now available correlating environmental factors, including water temperature, light intensity and photoperiod with conchospore production and release and seasonal occurrence of the blade phase (Conway and Cole 1977, Kapraun and Lemus 1987, Waaland *et al.* 1990).

Karyological studies indicate a basic haploid chromosome number of n=3-4 for most *Porphyra* species (Cole 1990, Kapraun *et al.* 1991). As in the closely related genus *Bangia* (Cole *et al.* 1983, Gargiulo *et al.* 1991), some species of *Porphyra* are reported to include populations with different chromosome

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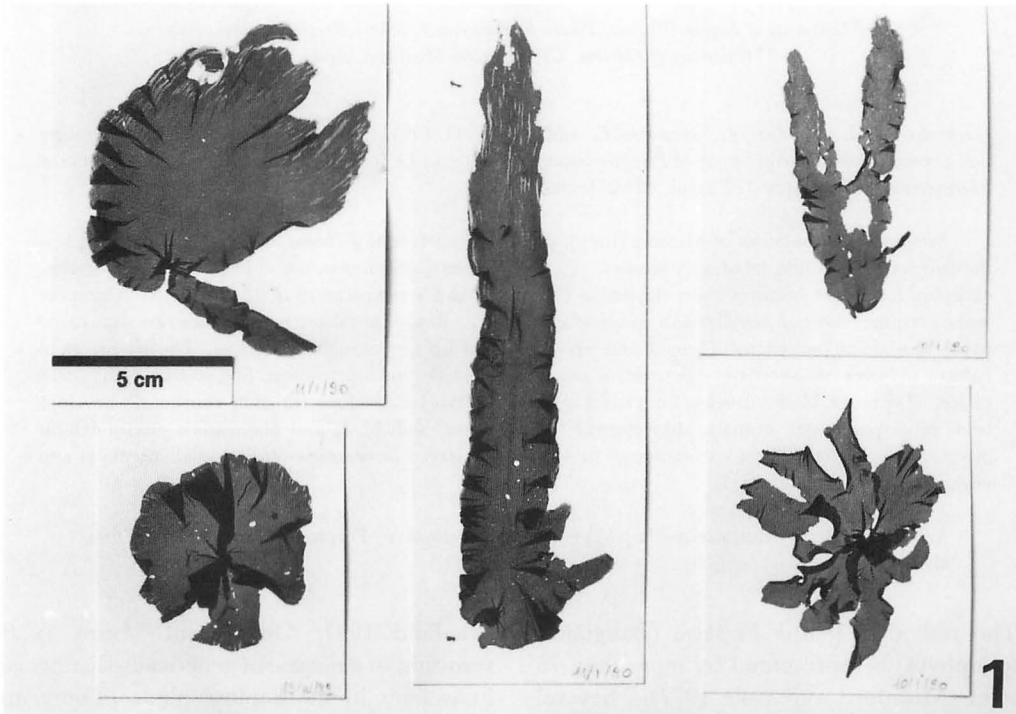
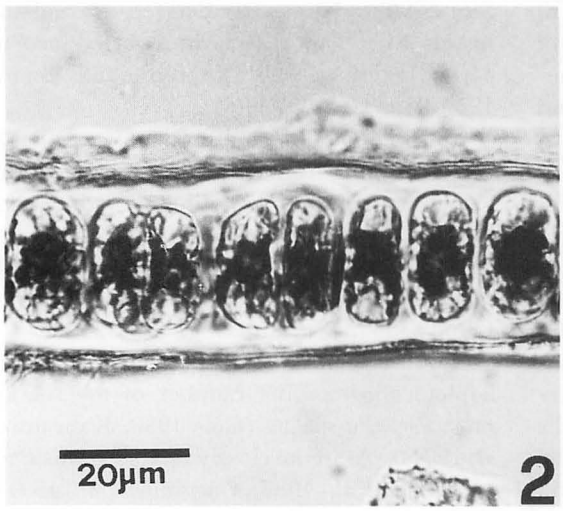
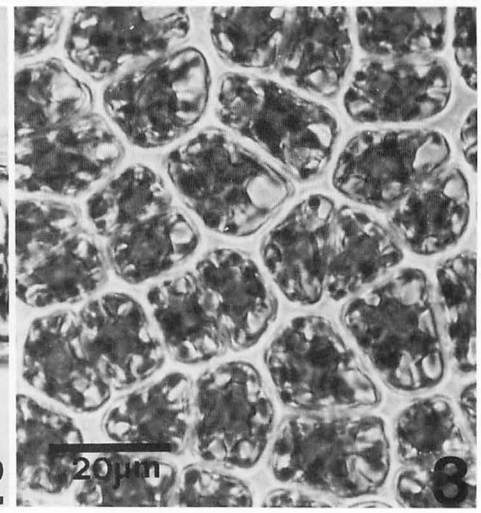
numbers (Kapraun *et al.* 1991).

Four species of *Porphyra* have been reported from the Mediterranean: *P. leucosticta* Thuret in Le Jolis, *P. linearis* Greville, *P. perforata* J. Agardh and *P. umbilicalis* (L.) J. Agardh (Hamel 1924, Lanfranco 1969). No published information is available for the life histories in controlled conditions, or on conchospore formation and release for Mediterranean isolates of any of these species. Karyological data for Mediterranean specimens are limited to chromosome numbers for the blade phase of three of these species (Kapraun and Freshwater 1987, Gargiulo *et al.* 1989).

This comunication presents the results of culture and karyological studies conducted to





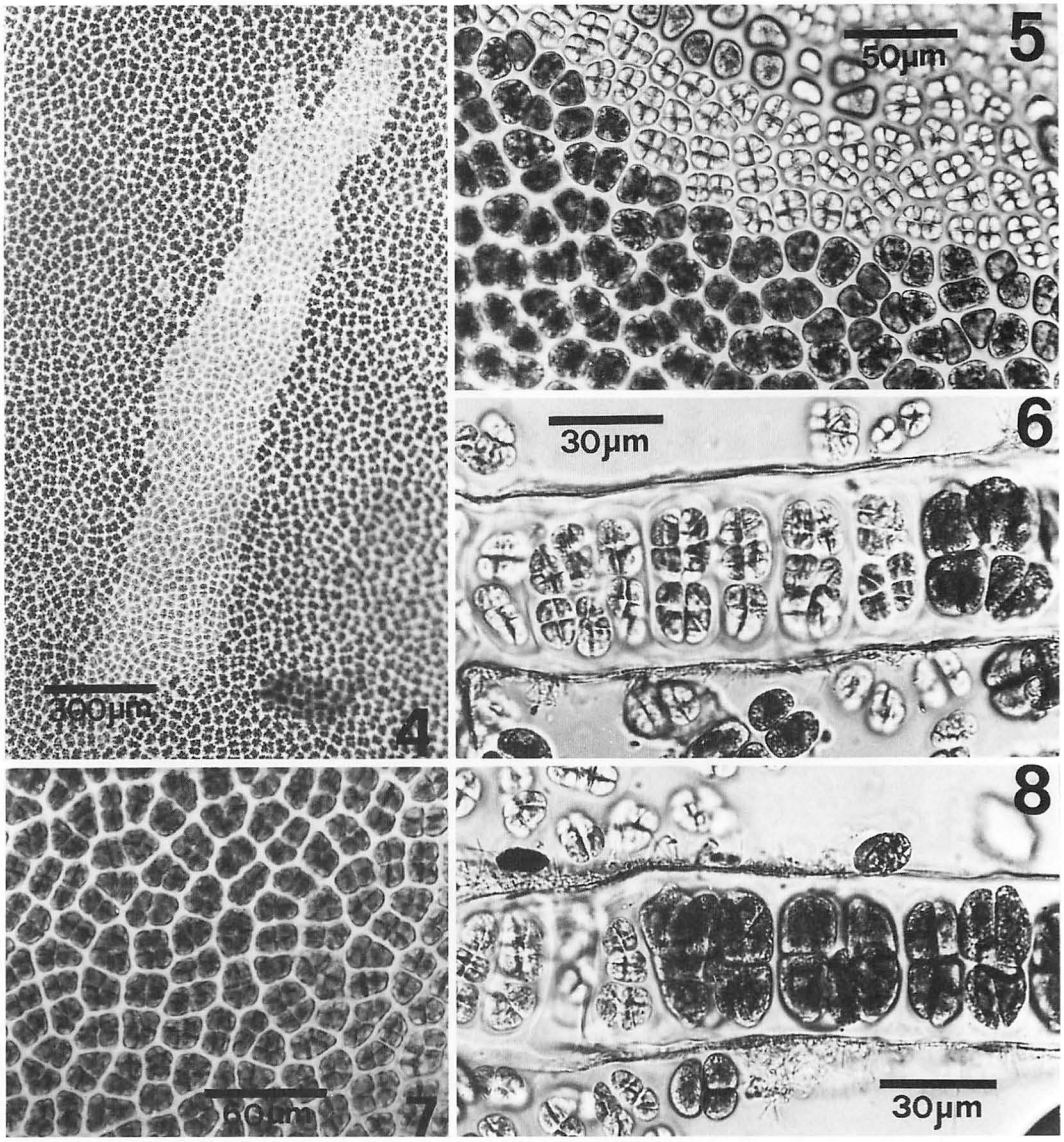


Figs. 1–3. *Porphyra leucosticta*. Fig. 1. Lanceolate, orbiculate or multilobed gametophytic thalli from the studied population. Fig. 2. Cross section of the monostromatic lamina. Fig. 3. Surface view of the lamina.

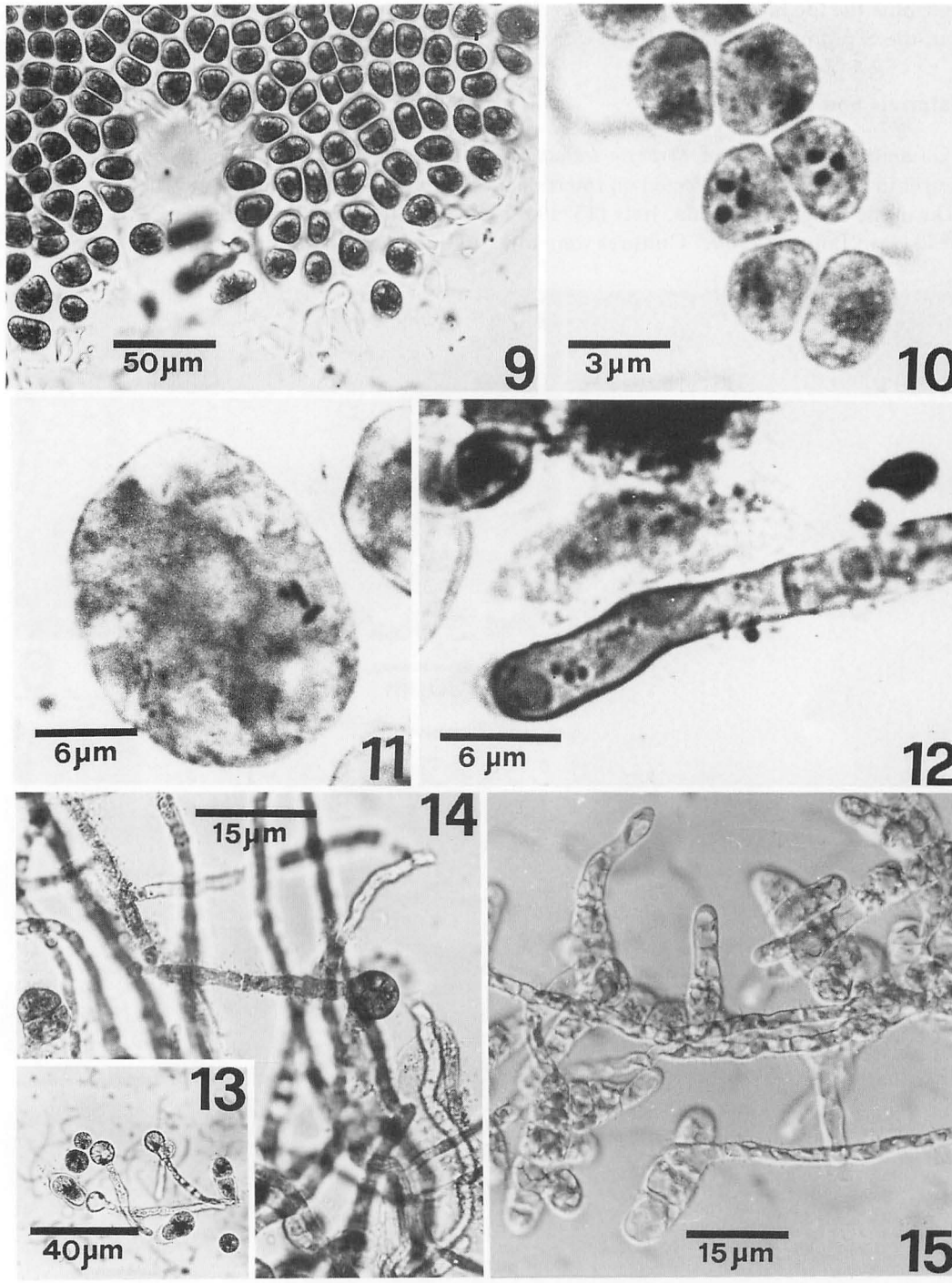
determine the life history of *Porphyra leucosticta* from the Strait of Messina, Italy.

Materials and Methods

Gametophytic plants of *Porphyra leucosticta* Thuret in Le Jolis were collected on intertidal rocks in the Strait of Messina, Italy (15°40′E, 38°30′N) in January 1990. Cultures were initiated from carpospores released from excised fragments of fertile fronds, gently brushed and washed with sterile seawater to reduce contaminants. Fragments were placed in Petri dishes (50 mm diam.) with sterile seawater. After spore release, excised fragments were removed and the spores incubated in a modified von Stosch's Medium (Guiry and Cunningham 1984), changed



Figs. 4–8. *Porphyra leucosticta*. Fig. 4. An elongate area of gametophytic thalli where spermatangia are differentiated; the other cells are carposporangia. Fig. 5. Surface view of the lamina with spermatangia. Fig. 6. Cross section of a fertile thallus, where spermatangia are visible. Fig. 7. Surface view of carposporangia. Fig. 8. Cross section of a fertile thallus with carposporangia.



Figs. 9–15. *Porphyra leucosticta*. Fig. 9. Surface view of marginal monosporangia. Figs. 10–12. Haploid mitotic nuclei with n=3. Fig. 10. Two spermatangia in late prophase. Fig. 11. Vegetative thallus cell. Fig. 12. Vegetative conchocelis cell. Fig. 13. Early stages of unipolar germination of carpospore. Fig. 14. Conchocelis thallus originated from unipolar germination of carpospores. Fig. 15. Conchosporangia differentiated on conchocelis filaments.

Table 1. Effect of photoperiod and temperature on the carpospore germination of *Porphyra leucosticta* at 50 $\mu E \cdot m^{-2} \cdot s^{-1}$

Photoperiod (h light : h dark)	Temperature (°C)	Carpospore germination
8 : 16	10	+
	15	+
	18	+
	22	+
	25	−
12 : 12	10	+
	15	+
	18	+
	22	+
	25	−
16 : 8	10	+
	15	+
	18	+
	22	+
	25	−

weekly. About twenty globose tufts of *conchocelis* filaments were allowed to grow in each dish, and three dishes where used in each experiment.

A cross-gradient incubator was used to test growth and reproduction of conchocelis filaments in different combinations of light and temperature. Cool white OSRAM fluorescent lamps produced a photon flux density at the surface of cultures between 5 and 70 $\mu E \cdot m^{-2} \cdot sec^{-1}$, measured with a LICOR Quantum radiometer model LI 185 A. Light regimes were: 8L : 16D; 9L : 15D; 12L : 12D; 16L : 8D. Experimental temperatures ranged between 10 and 28°C.

Field observations on local marine environmental conditions and on phenology of *P. leucosticta* were carried out weekly during three years (1990-92).

Chromosome counts were obtained for cultured and field collected material after fixation in 3 : 1 ethanol : glacial acetic acid and aceto-orcein staining (Gargiulo *et al.* 1991).

Observations and photomicrographs were made on a Leitz Diaplan microscope equipped with a standard optical system and Nomarsky interferential contrast.

Observations

1. *Thallus morphology.* Gametophytic plants are monoecious; the blades are lanceolate to multilobed with ruffled margin, up to 15 cm, but umbilicate plants are also frequent (Fig. 1). The base of the blade is orbiculate with a tiny holdfast. Plants are reddish purple, with linear whitish patches where spermatangia are formed. The plants are saxicolous.

2. *Microscopic observations.* The blade is monostromatic (Fig. 2), in surface view cells appear roughly quadrangular 10–15 × 15–20 μm (Fig. 3). Spermatangia differentiate

Table 2. Effect of photoperiod and temperature on the formation of conchosporangia of *Porphyra leucosticta* at 50 $\mu E \cdot m^{-2} \cdot s^{-1}$

Photoperiod (h light : h dark)	Temperature (°C)	% of *Conchocelis* thalli producing sporangia
8 : 16	10	Filaments remain vegetative
	15	100% conchosporangia formed
	18	100% conchosporangia formed
	22	Filaments remain vegetative
12 : 12	10	Filaments remain vegetative
	15	15% conchosporangia formed
	18	15% conchosporangia formed
	22	Filaments remain vegetative
16 : 8	10	Filaments remain vegetative
	15	Filaments remain vegetative
	18	Filaments remain vegetative
	22	Filaments remain vegetative

Table 3. Effect of photoperiod and temperature on the conchospores release of *Porphyra leucosticta* at 50 $\mu E \cdot m^{-2} \cdot s^{-1}$

Photoperiod (h light : h dark)	Temperature (°C)	% of *Conchocelis* thalli releasing spores
8 : 16	10	No release
	15	No release
	18	70% spores released
	22	No release
9 : 15	10	No release
	15	No release
	18	100% spores released
	22	No release
12 : 12	10	No release
	15	No release
	18	No release
	22	No release
16 : 8	10	No release
	15	No release
	18	No release
	22	No release

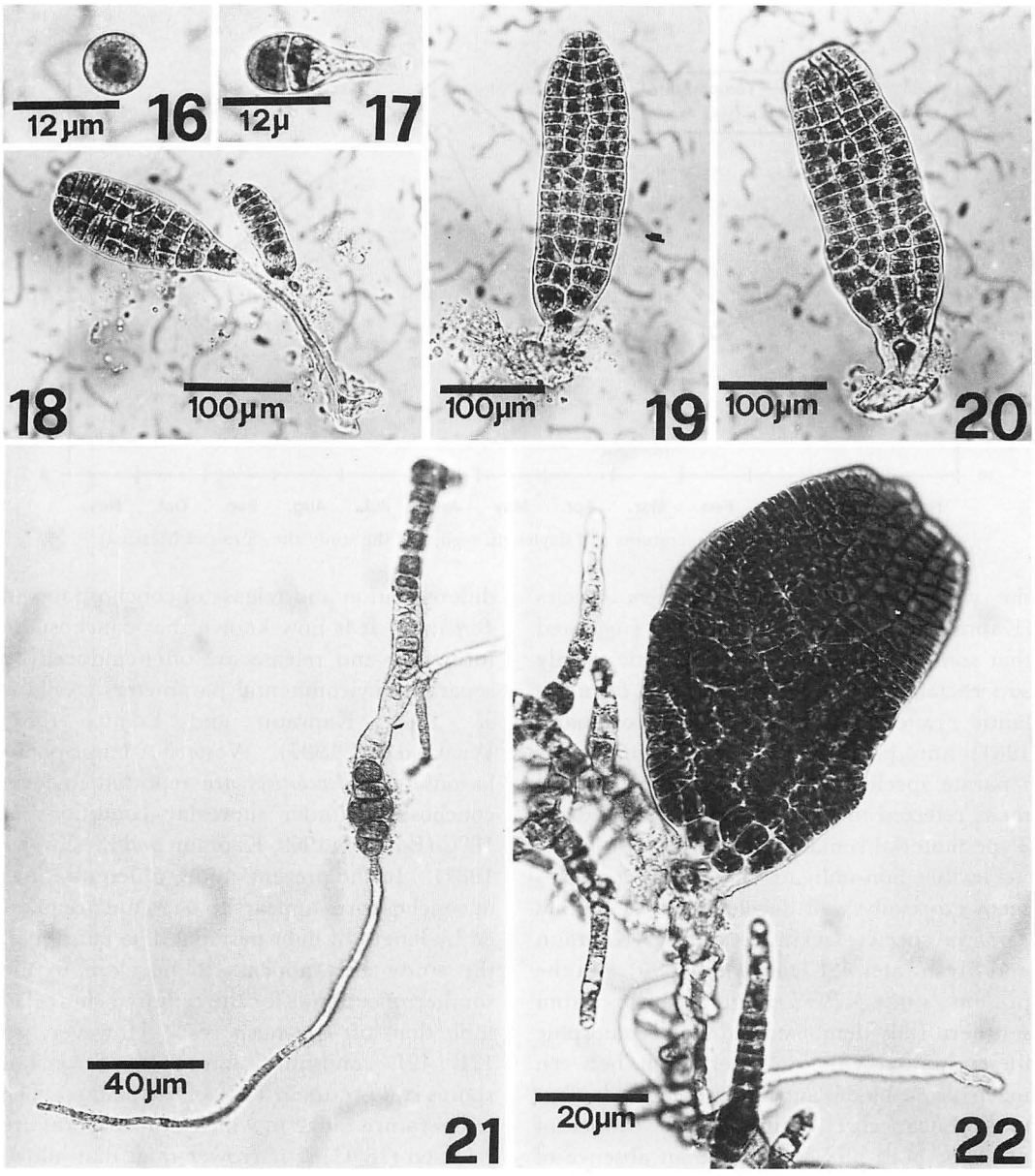
in the distal part of the blade in whitish stripes (Fig. 4) and marginal zones (Fig. 5). A single spermatangium (Figs. 5, 6, 8) is about 13×18 μm and forms 64 spermatia. Carpogonia are about 15×20 μm, formed adjacent to areas where spermatangia are differentiated. Developing carposporangia give rise to 8 carpospores (Figs. 5-8). Monospores are directly derived from vegetative cells on the margin of the blade (Fig. 9).

3. *Karyological observations.* Haploid chromosome numbers of $n=3$ were observed in spermatangia (Fig. 10), vegetative blade cells (Fig. 11) and conchocelis filaments (Fig. 12).

4. *Culture.* The fragments of leafy plants in culture released carpospores in all the tested conditions of light and temperature; the photoperiod had no influence on the release of carpospores. Germination of carpospores was observed 6 to 12 days from their release.

Unipolar germination of carpospores (Figs. 13, 14) results in the formation of branched filaments of the conchocelis stage which more or less adhere to the culture vessel or, more frequently, assume a free cushion shape. The effects of photoperiod and temperature on carpospore germination are shown in Table 1. Under optimal environmental conditions, the filaments differentiate conchosporangia (Fig. 15) that release conchospores. These germinate in a bipolar way forming new blades (Figs. 16-20). Under long-day conditions the *conchocelis* filaments never differentiated conchosporangia. The effects of photoperiod and temperature on the differentiation of cochosporangia on the filamentous plants are shown in Table 2. The release of conchospores was observed only under short-day regimes, and only at 18°C. The effects of photoperiod and temperature on the release of conchospores are shown in Table 3.

Formation of monospores on the conchocelis phase was not observed in these experiments. However, the unipolar germination of cells that are morphologically similar to conchospores resulted in conchocelis filaments (Fig. 21). In a few cases, the filaments formed irregular outgrowths lacking polarity (Fig. 22). These protothalli (*sensu* Cole and Conway 1980) soon disaggregate into single cells, which after bipolar germination, formed leafy gametophytic plants.



Figs. 16–22. *Porphyra leucosticta*. Figs. 16–20. Bipolar germination of carpospore and early formation of laminar gametophytic thallus. Fig. 21. *In situ* unipolar germination of a cell-like conchospore. Fig. 22. Formation of a protothallus from conchocelis filaments.

5. *Field observations.* The intertidal sea temperature where field observations were carried out ranged between 15°C in February and 25°C in August. The pattern of average temperatures as well as hours of light the year round are presented in Fig. 23.

Gametophytic blades appeared in November and were present until early June. Conchocelis filaments were not observed in nature.

Discussion

Porphyra leucosticta is reported to have one of

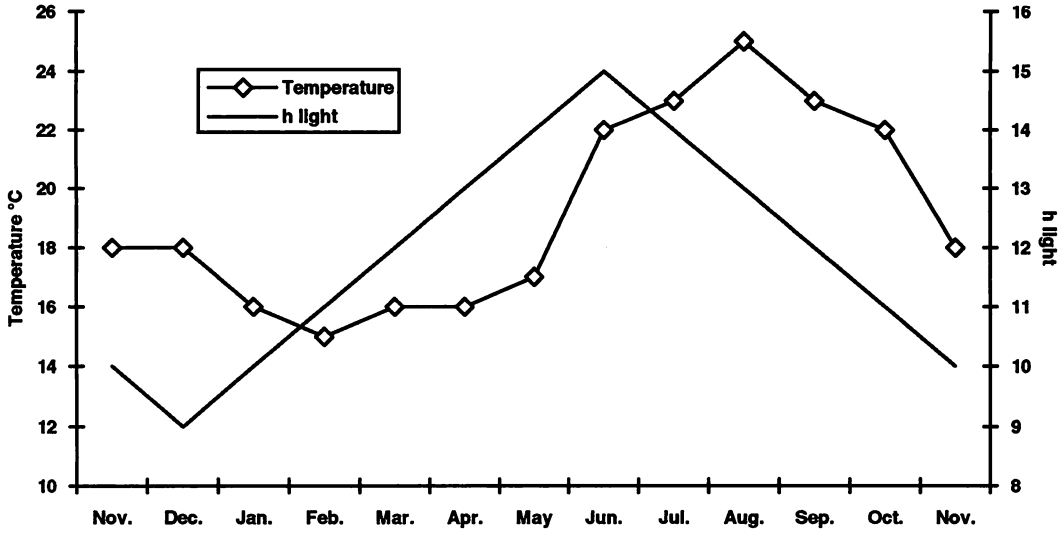


Fig. 23. Annual temperatures and daylength regime of the study site (Strait of Messina).

the widest ranges of any *Porphyra* species (Kapraun *et al.* 1991). It has been suggested that some of these regional populations only superficially resemble Type material from Atlantic France (Rosenvinge 1909, Kornmann 1961) and perhaps deserve recognition as separate species. The relationship of specimens referred to *P. leucosticta* in this study to Type material remains unknown.

Flexible non-obligate life history patterns seem especially well developed in temperate *Porphyra* species lacking sexuality (Kapraun and Freshwater 1987, Hawkes 1990). In the present study, *P. leucosticta* isolates from southern Italy demonstrated a heteromorphic life-cycle involving an alternation between macroscopic blades and a microscopic filamentous conchocelis. Chromosome counts of n=3 for both phases indicate an absence of sexuality and meiosis in these isolates. *Porphyra leucosticta* populations in North Carolina and Texas (Kapraun and Freshwater 1987, Kapraun *et al.* 1991), Uruguay (Coll and de Oliveira 1977a, b) and Helgoland (Kornmann 1961) are reported to have similar asexual life histories. In contrast, populations from Nova Scotia are reported to be sexual (Yabu 1978, Lindstrom and Cole 1992).

Kurogi (1959) first demonstrated the effects of temperature and photoperiod on the differentiation and release of conchospores in *Porphyra*. It is now known that conchospore formation and release are often induced by separate environmental parameters (Avila *et al.* 1986, Kapraun and Lemus 1987, Waaland *et al.* 1987). Western Atlantic populations of *P. leucosticta* are reported to form conchospore under short-day conditions at 18°C (Edwards 1969, Kapraun and Freshwater 1987). In the present study, differentiations of conchospores appears to be mainly regulated by length of light period. The latitude of the study area appears to be close to the southernmost limit for the required short-day induction of conchospores. However, in 12L : 12D conditions, formation of conchospores still occurs in 15% of the plants. The temperature range in which conchospores are released (18°C) is narrower than that allowing their formation (15-18°C). Water temperature in the intertidal in latitudes to the south of the study area becomes critical, often exceeding the temperature which permits differentiation of conchosporangia.

It is noteworthy that in culture, *P. leucosticta* demonstrated direct development of blades from the filamentous phase by differentiation of prothallus cells (Cole and Conway 1980). Germination of conchospore-like monospores resulted in the formation of new filamentous

thalli. The importance of these accessory reproductive modes to the persistence of *P. leucosticta* in the study area remains unknown.

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G. M. Gargiulo・F. De Masi*・G. Genovese*・G. Tripodi*：地中海産 *Porphyra leucosticta* Thuret in Le Jolis（紅藻，ウシケノリ目）の核学と生活環における温度と日長条件の効果について

イタリア，メッシナ海峡のアマノリ属のうち，これまで *Porphyra leucosticta* Thuret in Le Jolis として扱われてきた種につき野外及び室内での研究を行った。本種は照度・温度勾配装置による培養の結果，殻胞子の形成には短日条件 (9L : 16D) と 15–18°C の温度条件を必要とすることが明らかになった。また，殻胞子の放出には 18°C と短日条件が必要である。培養結果により調査地点での季節変化の説明を試みた。培養下では大型の葉状体と顕微鏡的な大きさのコンコセリスの間での交代がみられた。ふつう殻胞子から葉状体が発達する際には双極的な発芽をするのに対し，コンコセリスが発達する際には単極的な発芽をする。また，葉状体，糸状体のいずれも直接型の発芽をする。栄養的な葉状体，造精器，コンコセリスの栄養細胞では染色体数は n＝3 であった。(University of Reggio Calabria, Piazza S. Francesco 4, 89061 Reggio Calabria, Italy,* University of Messina, CP 58 98166 Messina S. Agata, Italy)

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