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Reproductive phenology of *Gigartina pacifica-ochotensis* and *Petrocelis* (Rhodophyta) in Oshoro Bay, Hokkaido*

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The seasonality of reproduction in *Gigartina pacifica-ochotensis* and *Petrocelis* sp. in Oshoro Bay, western coast of Hokkaido in Japan was investigated. *Gigartina* plants with mature cystocarps were found throughout the year. Nearly all of the carpospores cultured (65 isolates of 66 isolates) from the *Gigartina* gave rise to basal discs with upright *Gigartina* blades that reproduced by putative apomixis and only 1 isolate grew into *Petrocelis* crusts. The results of carpospore cultures from periodic sampling of individually tagged plants indicated that all the plants investigated were consistent in reproductive type. *Petrocelis* crusts showed a clear seasonal pattern in reproduction. Tetrasporangial primordia appeared during mid-October and early November. Mature tetrasporangia were found from late November to early February, occasionally mid-April. Cultured tetraspores from *Petrocelis* gave rise to dioecious *Gigartina* plants. The female plants produced cystocarps only in the presence of male plants with spermatia, and not in their absence.

Key Index Words: Gigartina; *Gigartinaceae*; G. ochotensis; G. pacifica; *life history*; Mastocarpus; Petrocelis; *phenology*; *reproduction*; *Rhodophyta.*

The species of *Gigartina* subgenus *Mastocarpus* possess both sexual and putatively apomictic life histories (WEST 1972, CHEN *et al.* 1974, POLANSHEK and WEST 1975, 1977, MASUDA and UCHIDA 1976, WEST *et al.* 1977, RUENESS 1978, WEST *et al.* 1978, DION and DELÉPINE 1979, MASUDA and KUROGI 1981). The sexual life history involves the alternation of foliose dioecious gametophytes (*Gigartina*) with a crustose tetrasporophyte (*Petrocelis*), whereas the putatively apomictic life history involves only cystocarpic plants. The relationship of the sexual and apomictic plants has not been resolved.

This study was undertaken to investigate: (1) the seasonality of reproduction in both sexual and apomictic *Gigartina* and in *Petrocelis*, and (2) whether seasonal changes between sexual and apomictic reproduction occur in the same plant. The investigation took place in Oshoro Bay, western coast of Hokkaido, where the reproduction of individually tagged plants was closely followed. The results of our investigation of reproductive phenology in *Gigartina* and *Petrocelis* are presented in this paper. The detailed results of the culture experiments will be presented in a separate paper together with information on other *Gigartina* and *Petrocelis* populations growing in northern Japan.

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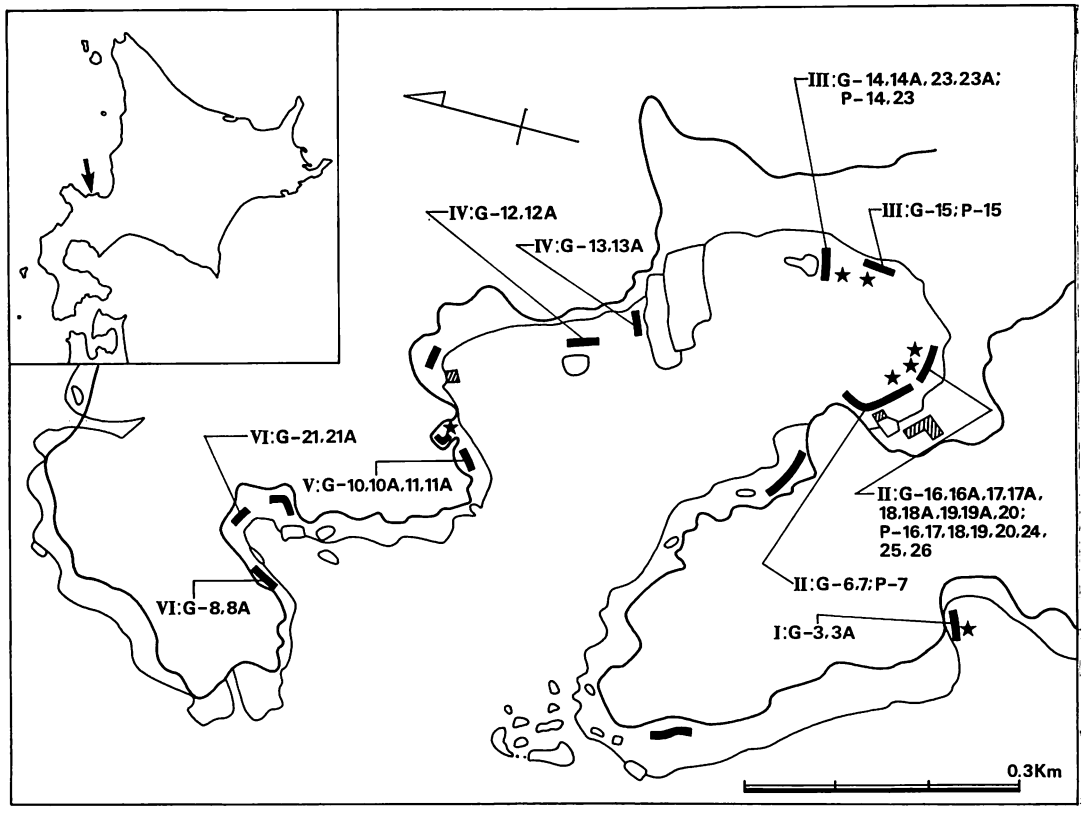
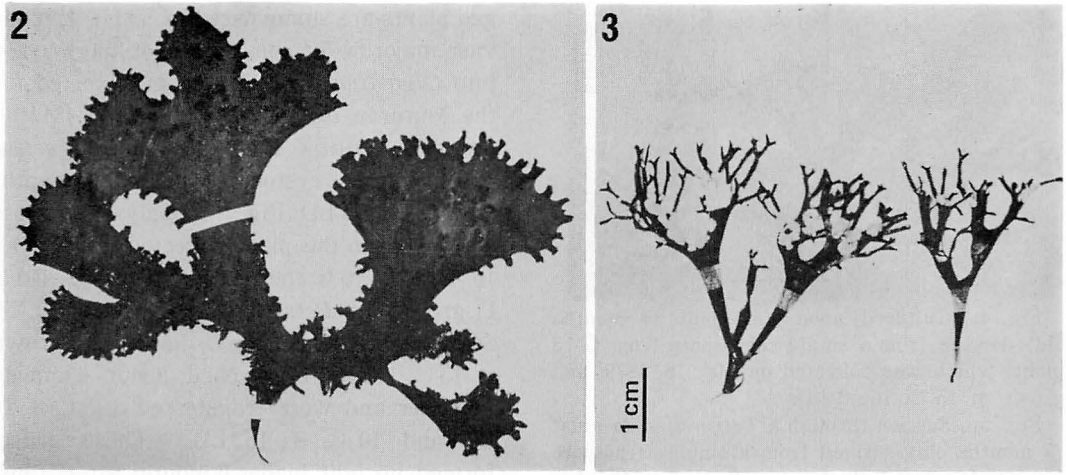


Fig. 1. Map of Oshoro Bay, showing horizontal distribution of *Gigartina pacifica-ochotensis* and *Petrocelis*, collection sites and individually tagged plant number. I-VI, collection sites; ■, G, *Gigartina*; ★, P, *Petrocelis*; arabic numerals, initially tagged plant number; numerals immediately followed by A, newly tagged plants in the course of investigation.

Materials and Methods

Field investigations on the distribution of *Gigartina* and *Petrocelis* in Oshoro Bay (43°13′N, 140°51′E) were undertaken in April 1979 and the survey sites were recorded on a map (Fig. 1). There are two types of *Gigartina* populations; those (I, II, III and V) associated with *Petrocelis* crusts and those (IV and VI) not associated with the crusts. In the six populations 17 plants of *Gigartina* and 9 plants of *Petrocelis* were individually tagged. Each plant was marked with a nail hammered into rock near the plant and then tagged with colored tape. Three more *Petrocelis* plants were added to the survey after December 1979. However, of 17 individually tagged *Gigartina* plants, only 3 plants (G-6, G-7 and G-20 in Table 1) were traced throughout the study. The others were lost by various disturbances. After a plant loss, another plant growing near the missing plant was tagged anew (Fig. 1, Table 1). One or two blades of the *Gigartina* plants and a piece of the *Petrocelis* crusts were sampled monthly or twice a month and reproductive structures were examined from April 1979 to March 1980. When spores were released, laboratory culture experiments were initiated to ascertain the fate of the sporelings.

Unialgal cultures were established as follows. Fertile *Gigartina* blades or *Petrocelis* crusts were cleaned by Kimwipes and washed in sterile seawater. From August 1979 *Gigartina* blades were surface sterilized



Figs. 2-3. Herbarium specimens of *Gigartina*. 2. G-8 plant (*G. pacifica* form) collected on June 15, 1979. 3. G-11A plant (*G. ochotensis* form) collected on January 10, 1980. Scale in Fig. 3 applies also to Fig. 2.

in 2% NaOCl/sterile seawater for 1-2 min, and rinsed 1 min in sterile seawater. Liberated spores were inoculated into several drops of PES culture medium on slide glasses placed on the bottom of Petri dishes (90 mm×20 mm) using finely-drawn glass capillary pipettes. The Petri dishes were then placed under culture conditions. The spores attached to the slide glasses 24-48 hr after inoculation, at which point about 50 ml of medium were introduced into the Petri dishes. After 7 days, the slides were transferred to individual glass storage jars (65 mm×80 mm) containing 200 ml of medium. One ml of 0.1% GeO_2 solution was added to cultures contaminated with diatoms (WEST 1972). The cultures were placed in freezer-incubators (2000-3000 lux) at 15 C, 16 : 8 LD (light-dark cycle), 10 C, 16 : 8 LD, 10 C, 8 : 16 LD and 5 C, 8 : 16 LD. The culture medium was changed monthly.

Observations

Gigartina

Gigartina grows on rocks in sheltered places in Oshoro Bay (Fig. 1). Plants with cystocarpic papillae were present throughout the year, but plants with spermatangial sori were never observed. Two forms of plants with cystocarpic papillae could be distinguished. The plants with papillae both on the blade surface and margin (*G. pacifica* form) luxuriated in summer and decreased in number in winter (Fig. 2). On the other hand, the plants with papillae only on blade margins (*G. ochotensis* form) predominated in winter and diminished in summer (Fig. 3). However, it was frequently observed that both forms arose from the same basal disc at the same time. Both forms reached a maximum height (5-7 cm) in summer. *Gigartina pacifica* KJELLM. and *G. ochotensis* (RUPR.) RUPR. have been reported in Oshoro Bay (TOKIDA and MASAKI 1959). However, our field observations indicate that the aforementioned two forms can not be disitnguished at the species level. At present we are unable to assess the influence of environmental factors on the position of cystocarpic papillae of *Gigartina*. The taxonomic relationship of *G. pacifica* and *G. ochotensis* will be discussed in another paper on the basis of hybridization experiments.

Carpospore liberation occurred throughout the year in the Oshore *Gigartina* populations, although not all plants traced continued to release carpospores. The results of culture experiments with carpospores from periodic sampling of individually tag-

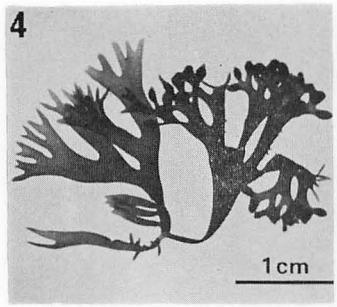


Fig. 4. Cultured apomictic plant (8 months old) derived from a single carpospore from G-13 plant, which was collected on May 16, 1979, and grown at 15 C, 16 : 8 LD.

Fig. 5. Section through a *Petrocelis*-like crust (7 months old) derived from a single carpospore from G-16 plant, which was collected on October 16, 1979, and grown at 15 C, 16 : 8 LD.

ged plants are summarized in Table 1. The vast majority of the carposporelings grew into *Gigartina* plants again as reported for the Muroran isolate of *G. ochotensis* (MASUDA and UCHIDA 1976). These *Gigartina* plants formed cystocarps after 6-9 months at 15 C, 16 : 8 LD (Fig. 4). Only one isolate derived from the plant collected on October 16, 1979 (G-16 in the II population, see Table 1) grew into *Petrocelis*-like crusts (Fig. 5). Seven-month-old *Petrocelis*-like crusts grown at 15 C, 16 : 8 LD reached about 6 mm in diameter and were transferred to 5 C, 8 : 16 LD and 10 C, 8 : 16 LD. These crusts formed upright blades 6 months after transfer. Sections of the crusts showed no signs

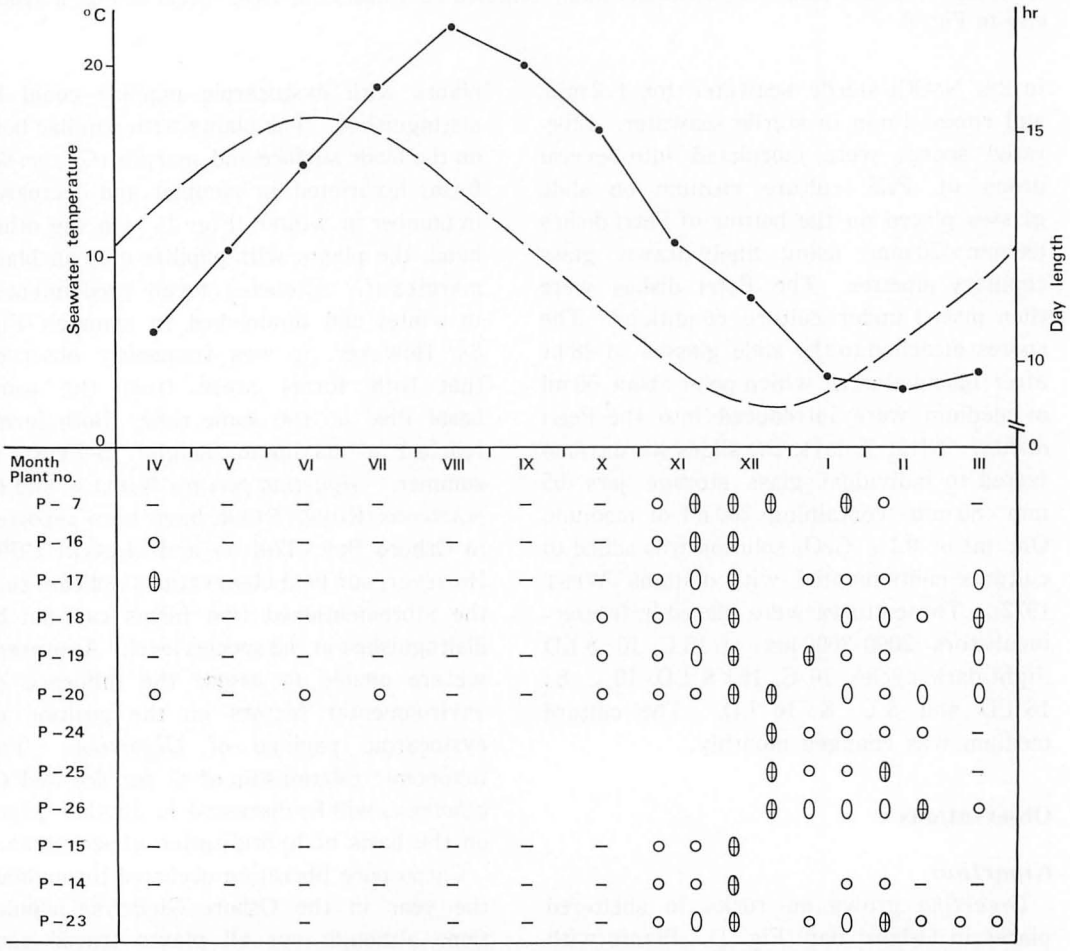
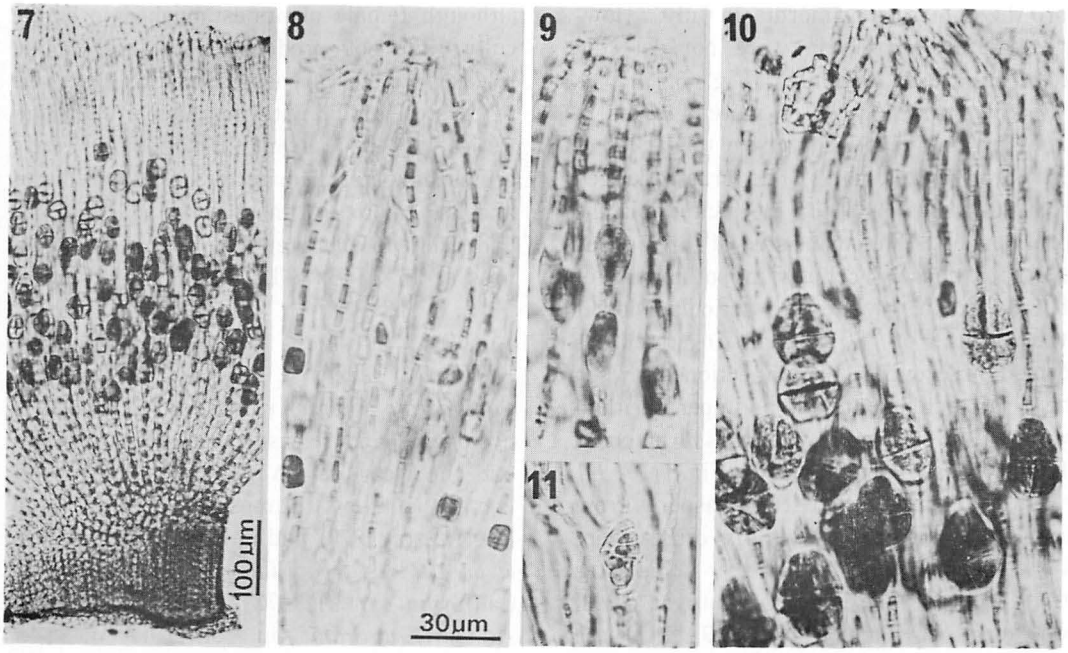


Fig. 6. Summary of reproductive phenology of individually tagged *Petrocelis* plants from April 1979 to March 1980 correlated to average monthly surface seawater temperature (solid line) and day length (dashed line). ○, tetrasporangial primordia (see Fig. 8) ; 0, young undivided tetrasporangia (see Fig. 9) ; ⊕, mature tetrasporangia (see Fig. 10) ; −, no reproductive structures; blank, no sampling.



Figs. 7-11. Sections through *Petrocelis* crusts. 7. Non-tagged plant collected on December 13, 1979. Note that the hypothallium is incomplete; 8. P-19 plant collected on November 10, 1979, showing tetrasporangial primordia; 9. P-20 plant collected on January 23, 1980, showing young undivided tetrasporangia; 10. P-19 plant collected on December 13, 1979, showing mature tetrasporangia; 11. P-7 plant collected on February 9, 1980, showing a tetrasporangium germinating *in situ*. Scale in Fig. 8 applies also to Figs. 9-11.

of tetrasporangia. We are uncertain whether *in situ* germination occurred in these crusts. Five-month-old blades were shifted to 15 C, 16 : 8 LD. None of the blades reaching up to 2 cm in length has produced any reproductive structures as of May 1981.

We also cultured 11 isolates derived from 11 non-tagged *Gigartina* plants, whicn were in association with *Petrocelis* crusts, during the study. All the carposporelings grew into *Gigartina* plants that reproduced by carpospores.

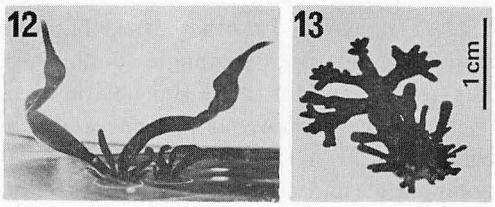
Petrocelis

Petrocelis plants also grow in sheltered places in Oshoro Bay (Fig. 1). Tetrasporangial primordia which were deeply pigmented, single and intercalary on perithallium filaments appeared during mid-October and early November (Figs. 6, 8). Tetrasporangia became mature after late November and in all the *Petrocelis* crusts examined the sporangia reached maturity in December (Figs. 6, 10). Mature cruciate tetrasporangia measured 18-28 μm in diameter and 25-38 μm in length (Fig. 10) and released tetraspores averaging 19μm (15-25μm) in diameter. From January to March new tetrasporangial primordia and young undivided sporangia (Fig. 9) appeared in other sori, but the number of mature sporangia diminished. Only the P-17 plant in the II population (see Fig. 1) formed a few mature sporangia in mid-April. From January to April tetrasporangia germinated *in situ* frequently (Fig. 11). This might indicate that tetraspore liberation came toward the end. No upright thalli were found on the *Petrocelis* crusts of which tetrasporangia germinated *in situ*. From May to September no tetrasporangial primordia were present except in the P-20 plant in the II population. This plant bore a few tetrasporangial primordia in June and

bore the numerous primordia in July. However, mature sporangia were not detected from August to November (Fig. 6).

Fertile *Petrocelis* crusts were 4-15 cm in diameter and yellowish brown to dark reddish brown in color. The crusts ranged from 300 μm to 1100 μm in thickness in the soral portion. They consisted of three tissue layers: a densely cellular hypothallium of 100-380 μm in thickness, a reticulate lower perithallium of 80-400 μm in thickness formed by secondary pit connections between the vertical filaments and an upper perithallium of 120-500 μm in thickness composed of loosely coherent filaments (Fig. 7).

Culture experiments with tetraspores from individually tagged *Petrocelis* crusts were started on December 14, 1979 (2 isolates from the P-17 and P-18 plants at the II population), December 15, 1979 (1 isolate from the P-23 plant at the III population), December 19, 1979 (4 isolates from the P-20, P-24, P-25 and P-26 plants at the II population) and February 10, 1980 (1 isolate from the P-23 plant). The tetraspores germinated in culture and grew into discoid thalli. After 2 months, upright thalli arose from those discs which reached 800-900 μm in diameter at 15 C, 16 : 8 LD. The upright thalli grew into dioecious *Gigartina* blades which reached reproductive maturity after 6 months (Figs. 12, 13). Mature cystocarps appeared in female gametophytes 50 days after starting mixed cultures of female and male plants,



Figs. 12-13. Cultured gametophytes derived from single tetraspores of field collected *Petrocelis*. 12. Male gametophyte (6 months old) from P-18 plant, which was collected on December 13, 1979, and grown at 15 C, 16 : 8 LD; 13. Female gametophyte (6 months old) from P-26 plant, which was collected on December 19, 1979, and grown at 15 C, 16 : 8 LD. Scale in Fig. 13 applies also to Fig. 12.

although female plants established in single culture did not produce cystocarps. They released viable carpospores which gave rise to crustose thalli with anatomical characteristics of the parent *Petrocelis*. These crusts have reached up to 3 mm in diameter and 300 μm in thickness at 10 C, 16 : 8 LD and 6 mm in diameter and 350 μm in thickness at 15 C, 16 : 8 LD as of May 1981.

Discussion

Gigartina plants in Oshoro Bay form cystocarps and release carpospores throughout the year as do *G. stellata* (STACKH.) BATT. in New Hampshire (HEHRE and MATHIESON 1970) and in Nova Scotia (CHEN *et al.* 1974), *G. papillata* (C. AG.) J. AG. in California (WEST 1972) and G. *jardinii* J. AG. (=*G. agardhii*) in California (WEST *et al.* 1978). The results of culture of these carpospores indicate that the vast majority of the sporelings reproduce by putative apomixis. Sixty-five of 66 isolates derived from 25 individually tagged plants and 11 non-tagged plants reproduced by apomixis and only 1 isolate reproduced sexually. Thus, apomictic *Gigartina* plants predominate in Oshoro Bay. However, it is curious that the sexual *Gigartina* plants are few in number in spite of the occurrence of its sporophytic *Petrocelis* crust. Although *Petrocelis* crusts in Oshoro Bay are not common, we have cultured carpospores from *Gigartina* plants associated with the crusts.

WEST *et al.* (1978), who first attempted to culture *Gigartina* carpospores and blade tips from periodic sampling of individually tagged plants, reported that most plants of *G. jardinii* were consistent and predictable in the nature of their reproduction, but one plant produced carpospores giving rise to both *Petrocelis*-like crusts and discs with blades at the same time. Furthermore, they reported that the blade tips formed procarpic papillae at one time and cystocarpic papillae were produced at another time. The results of our carpospore cultures from periodic

Table 1. Summary of reproductive phenology of individually tagged *Gigartina* plants from April 1979 to March 1980 and of their carpospore cultures. +, carpospores released; −, carpospores not released; v, vegetative; G, carposporelings giving rise to basal discs with *Gigartina* blades; P, carposporelings growing into *Petrocelis* crusts; blank, no sampling.

Plant no. \ Month	IV	V	VI	VII	VIII	IX	X	XI	XII	I	II	III
G-3	+				−	−*						
G-3A								+	G	G	−	
G-6	+		+	+	G	−*	v		G	G	G	
G-7	+		+	+	−	−*		G	G	G	+	G
G-16	v	v	v	−	−	−*	P					
G-16A								+				
G-17	v		v	+	G	−*						
G-17A							G					
G-18	v		G	+	+	−*						
G-18A							G	G	v	v		
G-19	v	v		+	−	−*						
G-19A										v	+	G
G-20	−	−		+	−	−*	+	G	G	G	G	G
G-15	G	+	+	+	+	−*						
G-14	+	G	−	+		−*						
G-14A								v	−	G	G	
G-23	v	v			+	−*	+					
G-23A									G	G	G	G
G-13	+	G		−		−*	G	G				
G-13A									G	G	G	G
G-12	−		−	−								
G-12A							−	G	G	G	G	G
G-21	v	+	G	−	−	v						
G-21A							v	−				
G-8	G	G	G	+		−*						
G-8A									−	−		
G-11	v		G	+	+	−*	G	G				
G-11A									G	G	G	
G-10	G		+	+	+	−*						
G-10A							v	−	G	G		

* Carpospore liberation may have been inhibited by a high concentration of NaOCl in seawater.

sampling of individually tagged plants indicate that all the plants investigated are consistent in reproductive type. However, we did not culture all the carpospores liberated and moreover, we were unable to obtain viable carpospores from the plants collected in September 1979 because of strong surface sterilization by NaOCl seawater solution (Table 1).

Our periodic examination of *Petrocelis* crusts in Oshoro Bay indicate that the crusts show a clear seasonal reproductive

pattern (Fig. 6). This may correlate with day length and seawater temperature. The formation of tetrasporangial primordia began during mid-October and early November, when the day length was about 10-11 hr and the seawater temperature was about 10-16 C. Tetrasporangia reach maturity from late November to early February (sometimes to April) when the day length was about 9-10 hr and the seawater temperature was about 3-8 C. FUNANO (1972) reported the occurrence of tetrasporangia in unidentified species of *Petrocelis* at Muroran, south coast of Hokkaido in Japan, from December to February. In describing *Petrocelis franciscana* (=*P. middendorffii*) from California, GARDNER (1917) stated that the crusts bear the tetrasporangia during December, January and February. HEHRE and MATHIESON (1970) reported the reproductive periodicity of *P. middendorffii* in New Hampshire. Its tetrasporangia are present from October to June. In laboratory culture experiments, *P. middendorffii* became reproductive at 10-12 C, 8: 16 LD (POLANSHEK and WEST 1975) or 15 C, 8: 16 LD (POLANSHEK and WEST 1977), and *Petrocelis* crusts derived from carpospores of *Gigartina ochotensis* reached repreductive maturity at 5 C, 8: 16 LD (MASUDA and KUROGI 1981). Thus, the tetrasporogenesis of *Petrocelis* may be induced by short days and relatively low temperatures. Several *Petrocelis* crusts derived from cultured *Gigartina* were shifted to 10 C, 8: 16 LD and 5 C, 8: 16 LD in May 1981 for further investigation.

Three species of *Petrocelis* with intercalary solitary tetrasporangia are known: *P. cruenta* J. AG., type species, *P. middendorffii* (RUPR.) KJELLM. and *P. ascendens* DAWS. *P. cruenta* is the naturally occurring tetrasporophyte of *Gigartina stellata* (WEST *et al.* 1977) and *P. middendorffii* is that of *G. papillata* (POLANSHEK and WEST 1977). Oshoro *Petrocelis* crusts examined are similar to both *P. middendorffii* and *P. cruenta*. According to WEST *et al.* (1977), these two algae may be distinguished by hypothallium thickness. In specimens of *P. middendorffii*, overall thallus thickness ranges from 250 μm to 1100 μm, the hypothallium constituting less than a quarter of the total thickness of the crust, whereas in specimens of *P. cruenta* thallus thickness is 700-800 μm, the hypothallium constituting approximately half of the total crust thickness (WEST *et al.* 1977). In Oshoro specimens total crust thickness is 300-1100μm and the hypothallium constitutes one-forth to four-tenths of the total thickness of the crust. At present we are unable to determine the specific status of *Petrocelis* found in Oshoro Bay.

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大野幸正*・増田道夫・黒木宗尚：忍路湾における紅藻イボノリーホソイボノリと *Petrocelis* の生殖季節

イボノリーホソイボノリと *Petrocelis* の生殖季節を，標識を付けた個体を1年間追跡調査して明らかにした。イボノリーホソイボノリにおいては形成される果胞子の発芽体が *Petrocelis* になるか，あるいは再びイボノリーホソイボノリの個体になるかを，*Petrocelis* においてはその四分胞子の発芽体の正体を確認するために，得られた胞子からの培養実験を行なった。イボノリーホソイボノリでは成熟した嚢果が1年中みられた。同一個体が周年果胞子の放出を続けることは確認されなかったが，調査した個体のいずれかが常に果胞子を放出し，個体群全体としては周年果胞子放出がみられた。培養した66藻株のうち65藻株が無配生殖を行なう個体に生長し，わずか1藻株が *Petrocelis* になった。また同一個体において，無配生殖と有性生殖の季節的転換はみられなかった。*Petrocelis* では生殖季節に明瞭な周期性が認められた。四分胞子嚢原基は10月中旬～11月上旬に形成され始め，完熟した四分胞子嚢は11月下旬～2月上旬に多くみられた。四分胞子の発芽体は雌雄異株のイボノリーホソイボノリの個体に生長した。(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：158 東京都世田谷区玉川3丁目14-5　新日本気象海洋株式会社)