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A note on parthenogenesis in four species of Laminariales

Geeta BHARATHAN* and Iwao SHINMURA**

* *Research Center of Central Marine Fisheries Research Institute 29, Commander-in-Chief Road, Madras-600 105, India*
** *Kagoshima Prefectural Fisheries Research Station, Kinko-cho, Kagoshima-shi, 892 Japan*

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Observations on parthenogenesis were made on 4 species of Laminariales, viz. *Undaria pinnatifida*, *U. peterseniana*, *Ecklonia cava* and *E. kurome*. Much higher reproductive ability of oogonia was found in the isolated female gametophytes of *Ecklonia* than in those of *Undaria*. The parthenogenetic sporophytes were produced at a higher rate in *Ecklonia* than in *Undaria*. The proportion of the eggs attached on or detached from empty oogonium to their germlings was examined in each species.

Key Index Words: *Culture*; Ecklonia cava; Ecklonia kurome; *Laminariales*; *parthenogenesis*; Undaria peterseniana; Undaria pinnatifida.

Parthenogenesis in various species of the Laminariales has been reported by many workers (SCHREIBER 1930, TOKIDA and YABU 1962, YABU 1964, NAKAHARA and NAKAMURA 1973, SANBONSUGA and NEUSHUL 1978, NAKAHARA 1984). During experiments involving interspecific crossing in the genera *Undaria* and *Ecklonia*, some interesting differences were noted in the rate of parthenogenesis and other features of reproductive behaviour among these taxa. These observations are reported in this note.

Materials and Methods

The female gametophytes being maintained in culture at the Kagoshima Prefectural Fisheries Research Station were used in these experiments. Cultures had been derived from single zoospores. Species studied were *Undaria pinnatifida*, *U. peterseniana*, *Ecklonia cava* and *E. kurome* (Table 1).

Stock cultures were subcultured during October 1983. These fresh cultures were kept in a growth chamber at 23°C under long day conditions (14 hr L : 10 hr D) for a period of two weeks. The gametophytes were then homogenized and inoculated into petri dishes containing Miquel's medium (1/2 strength). The dishes were transferred to a growth chamber maintained at 18°C and short day conditions (10 hr L : 14 hr D). These are conditions that induce formation of gametangia in these species.

Observations were made daily to check the onset of maturity. Final observations involving the quantitative estimation of reproductive parameters such as gametangial formation and parthenogenesis were made 60 days after the initial cultures were started; that is, in December 1983.

Observations were made on gametophytes as well as on detached eggs and embryos. The following were counted: total oogonia (*t*); "vegetative" branches (*v*)—the latter included both truly vegetative branches and immature oogonia-like branches. Of total oogonia, counts were made of those branches still bearing eggs (*e*) or parthenogenetic sporophytes (*p*) or those that had shed their eggs and were thus empty (*r*). Ten random

Table 1. Mother plants and their notes.

Species	Habitat	Date of seeding zoospores	Date of separating male and female gametophytes
Undaria pinnatifida	Akune, Kagoshima Pref. Wild plant	Mar. 17, 1976	Aug. 1976
U. peterseniana	Wakamatsu, Nagasaki Pref. Wild plant	Jun. 5, 1976	Aug. 30, 1977
Ecklonia cava	Futae, Kumamoto Pref. Wild plant	Nov. 13, 1980	Aug. 28, 1981
E. kurome	Tsuno, Miyazaki Pref. Wild plant	Nov. 5, 1976	Aug. 31, 1977

fields of the microscope were examined in this fashion. Separate counts were made of eggs (*ed*) and parthenogenetic sporophytes (*pd*) among detached eggs and sporophytes. Fifteen such fields were counted. In both cases the total number of eggs and parthenogenetic sporophytes occurring in each field of the microscope were counted.

From the above counts the relative proportions of eggs and sporophytes were calculated and the following parameters estimated:

1. Reproductive ability was defined as the proportion of oogonia among all branches, both oogonial as well as "vegetative", i.e. $t/(v+t)$ expressed as a percentage.

2. Rate of parthenogenesis was obtained from two separate estimates as follows; the proportions of detached eggs, A ($A=ed/(ed+pd)$), and parthenogenetic sporophytes, B ($B=pd/(ed+pd)$), were calculated. Assuming then that *ed* and *pd* had been released from oogonia in the same proportions as indicated by A and B, the number of eggs actually released from the empty oogonia (r) was estimated. These estimated numbers were then summed with the numbers of observed eggs and parthenogenetic sporophytes, respectively, to obtain the actual total number of eggs or parthenogenetic sporophytes. The proportion of parthenogenetic sporophytes calculated in this manner was taken to be a measure of the rate of parthenogenesis.

Results

Reproductive ability: The production of oogonia was much higher in *Ecklonia*. Of the new branches formed, 56.8% in *E. cava* and 62.7% in *E. kurome* produced eggs after 51 and 44 days, respectively. The percentage was slightly lower in *U. peterseniana* (41.7%) and much lower in *U. pinnatifida* (24.6%) (Table 2).

Parthenogenesis: The rate of production of parthenogenetic sporophytes estimated from the data of Tables 2–4 was high in *E. cava* (66.1%), *E. kurome* (62.7%) and *U. pinnatifida* (58.4%), but was lower in *U. peterseniana* (30.0%) (Table 5). As to the eggs attached on or detached from the empty oogonium, the following proportions relating to their germlings were observed in each species. Among attached eggs and germlings, the proportion of eggs was high in *Undaria* (51.7% in *U. pinnatifida* and 63.6 % in *U. peterseniana*), while it was much lower in *Ecklonia* (30.7% in *E. cava* and 33.2% in *E. kurome*) (Table 3). Among detached eggs and germlings, the percentage of eggs was higher in *U. peterseniana* (73.1 %) than in other three species (36.7% in *U. pinnatifida*, 40.0% in *E. cava* and 44.4% in *E. kurome*) (Table 4). In *U. peterseniana*, *E. cava* and *E. kurome*, the proportion of eggs was higher in detached ones (Table 4) than in attached ones (Table 3). In *U. pinnatifida* alone, the proportion of eggs was lower in detached ones (36.7%) than in attached ones (51.7%) (Table 3, 4, 5).

Discussion

Reproductive ability: Of the four species studied, *U. pinnatifida* appears to be the least

Table 2. Reproductive ability in four species of Laminariales.

Species	Total oogonia t	Empty oogonia r	"vegetative" branches v	Reproductive ability $t/(v+t)$ %
Undaria pinnatifida	89	60	272	24.6
U. peterseniana	166	111	232	41.7
Ecklonia cava	254	88	193	56.8
E. kurome	346	126	206	62.7

Table 3. Production of eggs in four species of Laminariales.

Species	Oogonia-bearing eggs e	Oogonia-bearing parthenogenetic sporophytes p	Proportion of eggs $e/(e+p)$ %
Undaria pinnatifida	15	14	51.7
U. peterseniana	35	20	63.6
Ecklonia cava	51	115	30.7
E. kurome	73	147	33.2

Table 4. Production of parthenogenetic sporophytes in four species of Laminariales.

Species	Detached eggs ed	Detached parthenogenetic sporophytes pd	Proportion of eggs $A=ed/(ed+pd)$ %	Proportion of parthenogenetic sporophytes $B=pd/(ed+pd)$ %
Undaria pinnatifida	105	181	36.7	63.3
U. peterseniana	114	42	73.1	26.9
Ecklonia cava	292	438	40.0	60.0
E. kurome	252	315	44.4	55.6

Table 5. Rate of parthenogenesis in four species of Laminariales.

Species	Estimated no. from empty oogonia		Estimated total no.		Parthenogenesis
	Eggs $Ee=A\times r$	Parthenogenetic sporophytes $Ep=B\times r$	Eggs $e+Ee$	Parthenogenetic sporophytes $p+Ep$	$(p+Ep)/t$ %
Undaria pinnatifida	22	38	37	52	58.4
U. peterseniana	81	30	116	50	30.0
Ecklonia cava	35	53	86	168	66.1
E. kurome	56	70	129	217	62.7

prolific reproductively, its production of oogonia being much lower than the others. *U. peterseniana* was more prolific, although much less so than *Ecklonia* spp. It is possible that this feature is related to the ecological requirement of these species. *U. pinnatifida* has a very wide distribution with different phenology in the northern and southern parts of Japan where it occurs. It has a long gametophytic phase of 2-5 months lasting through summer. *U. peterseniana* has a much more restricted distribution, but also possesses a long gametophytic phase. The greater propensity for vegetative growth in *U. pinnatifida* and, to a smaller extent, in *U. peterseniana,* may perhaps be related to the long-lived nature of their gametophytes. *E. cava* and *E. kurome,* on the other hand, have a very short gametophytic phase, reproduction being initiated even in 2-4 celled gametophytes which are formed in autumn. A tendency to produce numerous oogonia in a very short time should be advantageous in such a situation. Perhaps such a tendency is reflected in the high rate of production of oogonia observed in the present study.

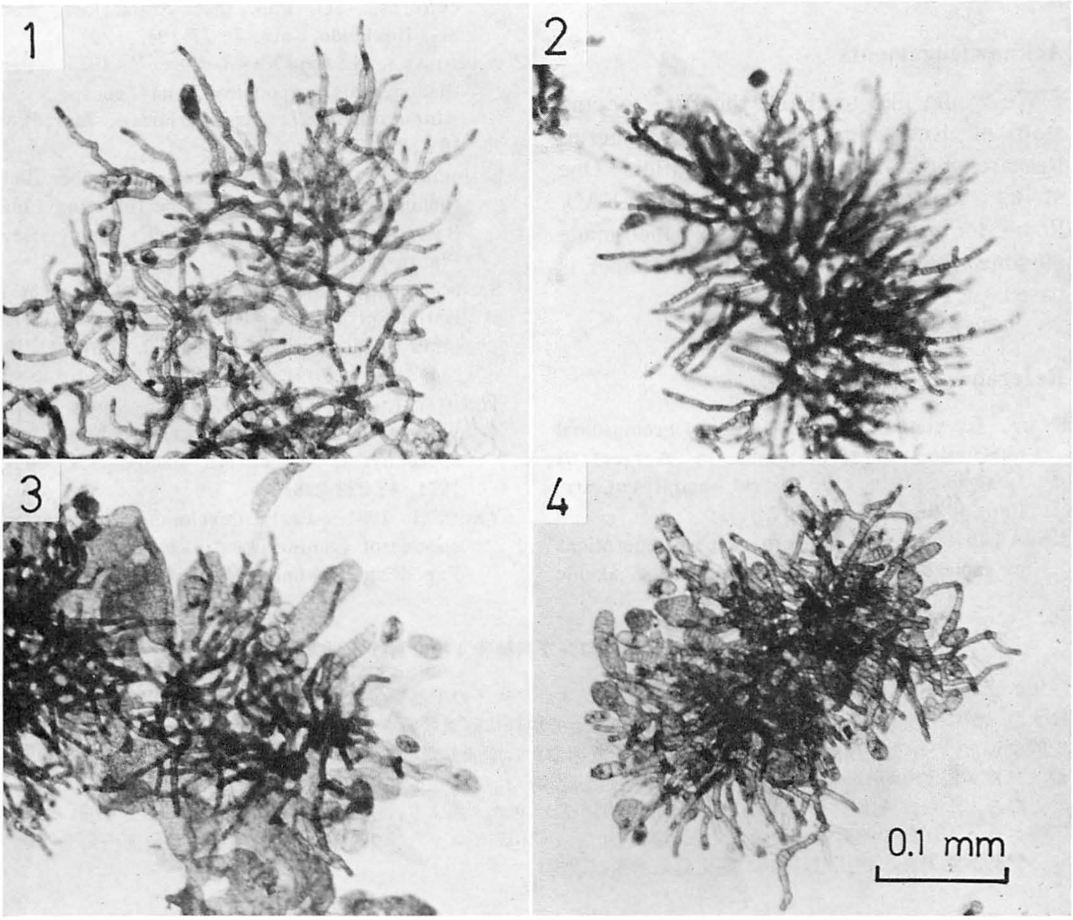
A possibility is that plasmogamy occurs in *U. pinnatifida* and, to a smaller extent, in *U. peterseniana,* as suggested for *Nereocystis luetkeana* by KEMP and COLE (1961). As noted also by SANBONSUGA and NEUSHUL (1978), few eggs are produced in *Macrocystis* and other kelps studied by them when only female plants are present. However, no comparative data for cultures containing both male and female gametophytes are available in the present study, and therefore no comment can be made on this point.

Parthenogenesis: Parthenogenesis may be considered in the above context. In the present study, *U. pinnatifida* and *Ecklonia* spp. showed a high rate of parthenogenesis, while *U. peterseniana* had a low rate. This indicates a greater sensitivity of the eggs of *U. peterseniana* which appear to lack the ability to develop themselves in the absence of fertilization. This greater sensitivity may be related to its more restricted distribution. In this species, as well as in the two species of *Ecklonia,* it was observed that the proportion of eggs was higher in the detached eggs and germlings than in the attached ones, indicating that eggs drop easier from the oogonium than do parthenogenetic sporophytes. In *U. pinnatifida,* however, the reverse holds true, and the percentage of 2-celled or more germlings actually increases in the detached eggs and germlings. It is probable that in this case many of the eggs divide after falling from the oogonium. This feature may reflect the more plastic nature of this species, which has a wide and varied distribution.

In *Undaria,* the relatively low rate of parthenogenesis was coupled with a moderate rate of production of oogonia in one case (*U. peterseniana*) and the relatively high rate of parthenogenesis was coupled with a low rate of reproduction in the other (*U. pinnatifida*). This means that only a very small number of parthenogenetic sporophytes are formed and remain attached to gametophytes (Figs. 1 and 2). In the two species of *Ecklonia,* on the other hand, the high rate of formation of reproductive organs as well as of parthenogenetic sporophytes results in the formation of several parthenogenetic sporophytes per gametophyte (Figs. 3 and 4).

It is possible to estimate from YABU (1964) the mean number of sporophytes produced per gametophyte in the different species as summarized in Table 6. While the culture conditions may not have been standardized and the observations were made after very long periods, it is nevertheless interesting to note some differences among the species studied. Even if comparisons of the present study with these figures are not strictly correct, the similarity between the different photographs may be noted (YABU 1964, plates XV-XVII and XXIV D).

The rate of parthenogenesis may be computed for *Alaria crassifolia* from Table 1 in NAKAHARA and NAKAMURA (1973). The figure obtained is 25.9%. Their observations were made 20 days after the transfer of



Figs. 1-4. Parthenogenetic sporophytes from unfertilized eggs in pure cultures of female gametophytes on Dec. 21, 1983 (60 days after the beginning of culture). Each gametophytes were maintained under the same conditions (18°C, 3-5 klux, 10 hr L : 14 hr D photoperiod). Parthenogenetic sporophytes are recognized much more in Figs. 3 and 4 than in Figs. 1 and 2. 1, *Undaria pinnatifida*; 2, *U. peterseniana*; 3, *Ecklonia cava*; 4, *E. kurome*.

Table 6. The number of sporophytes per female gametophyte in two species of Laminariales estimated from the data by YABU (1964).

Species	Sporophytes per female gametophyte	Observed after (days)
Laminaria japonica	15.0	129-300
Undaria pinnatifida	1.6	228

gametophytes to inducing conditions and this figure is therefore also not directly comparable with the results of the present study. However, it is clear from the photograph (NAKAHARA and NAKAMURA 1973, Fig. 2B) that the rate of production of attached parthenogenetic sporophytes is high in this species, resembling *Ecklonia*.

It is interesting to note that both *Alaria* and *Laminaria* have relatively short-lived gametophytes like *Ecklonia*. These admittedly preliminary observations appear to indicate distinct differences in reproductive strategy among different species of seaweeds. A detailed study of some of these aspects may be relevant to an understanding of the ecology and adaptive behaviour of these algae.

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バラタン，G.*・新村　巌**：コンブ科植物4種における単為発生について

コンブ科植物の4種，ワカメ，アオワカメ，カジメ，クロメの単為発生について観察した。4種の雌性配偶体は水温 18°C, 照度 3-5 klux, 日長 10L-14D のもとで純粋に隔離培養され，60日間卵および単為発生の形成状況を観察した。その結果，蔵卵器に付着した卵，蔵卵器から離脱した卵およびそれらの単為発生した胞子体の形成量はワカメ属よりカジメ属の方で高率を示す傾向が認められた。

このことから，配偶体の生殖能力は配偶体期間が長いワカメ属より，短期間のカジメ属の方がより強いことが示唆され，両属間の相対的特性であると推察された。(*105 インド　マドラス，中央海洋漁業研究所研究センター，**892 鹿児島市錦江町11-40，鹿児島県水産試験場)