Studies on the Scallop, Patinopecten yessoensis, in Sowing Cultures in Abashiri Waters : Reproductive Periodicity

Tohoku Journal of Agricultural Research
Vol. 35 No. 2-4, March 1985
Printed in Japan

Studies on the Scallop, *Patinopecten yessoensis*, in Sowing Cultures in Abashiri Waters ——Reproductive Periodicity*

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(Received, January 24, 1985)

Summary

Annual cycles of reproduction in the scallop, *Patinopecten yessoensis*, cultured by the sowing method in Abashiri waters, Hokkaido, are mainly described on the basis of monthly gonad indices and histological observations of gonadal tissues (oocyte size, nucleus diameter and number of genital tubules).

No appreciable difference in gonadal phases with seasons was recognized between the two areas, Abashiri Bay and the west of Point Notoro, in Abashiri waters.

The scallops developed gonads even during the drifting ice period and had no abnormal gonads, such as overripe gonads, which did occur in hanging cultured scallops.

With regard to gonadal growth distinct differences were observed between the scallops cultured by the sowing method in Abashiri waters and those cultured by the hanging method in Lake Saroma. However, there were no significant differences in the gonadal maturity.

The reproductive periodicity of the scallop cultured by sowing method in Abashiri waters could be differentiated into five successive stages: multiplicative (July to November), growing (October to April), mature (March to June), spawning (May to July) and recovery (June and July).

In Japan scallops are cultured in two ways, the hanging method and the sowing method. Recently, abnormal development of the scallop gonad in the hanging cultures has been reported by Mori and Osanai (1). They have suggested that the abnormal gonad might bring about the physiological disturbance and the mass mortality of this animal. In sowing cultures, mass mortality occurred once in Abashiri waters, Hokkaido, but the cause has remained unknown (2). In spite of the commercial importance of the scallop in sowing cultures, little information is available concerning its reproduction.

It is still unclear whether there is a difference in seasonal gonadal phases of

* The oral presentation of this report was given at the Autumn Meeting of Japan. Soc. Sci. Fish., Sendai, October 1, 1984.

scallops in Abashiri Bay and in the west of Point Notoro in Abashiri waters, though a significant difference in growth of the scallops in sowing cultures was recognized in these two areas (3).

Consequently, the present study was carried out in order to investigate the occurrence of abnormal gonads and to clarify the reproductive periodicity of the scallop in sowing cultures in Abashiri waters.

Materials and Methods

From September 1982 to June 1984, two to four-year-old scallops in sowing cultures were collected monthly from Abashiri Bay and from the west of Point Notoro in Abashiri waters. In addition, the hanging cultured scallops from Lake Saroma and the sowing cultured scallops from Lake Notoro were used for comparison (Fig. 1).

Gonad dissected from each animal was blotted dry and weighed. The gonad index was determined by a conventional formula:

$$\text{gonad index} = \frac{\text{gonad wet weight, g}}{\text{soft body wet weight, g}} \%$$

A piece of gonad near the nephridium of each specimen was prepared for histological examination by fixation in Bouin's fluid, dehydration in ethanol and embedding in paraffin wax (m.p. 58°C). Sections were cut at 6 μm and stained with Mayer's haematoxylin and 1% eosin-erythrosin.

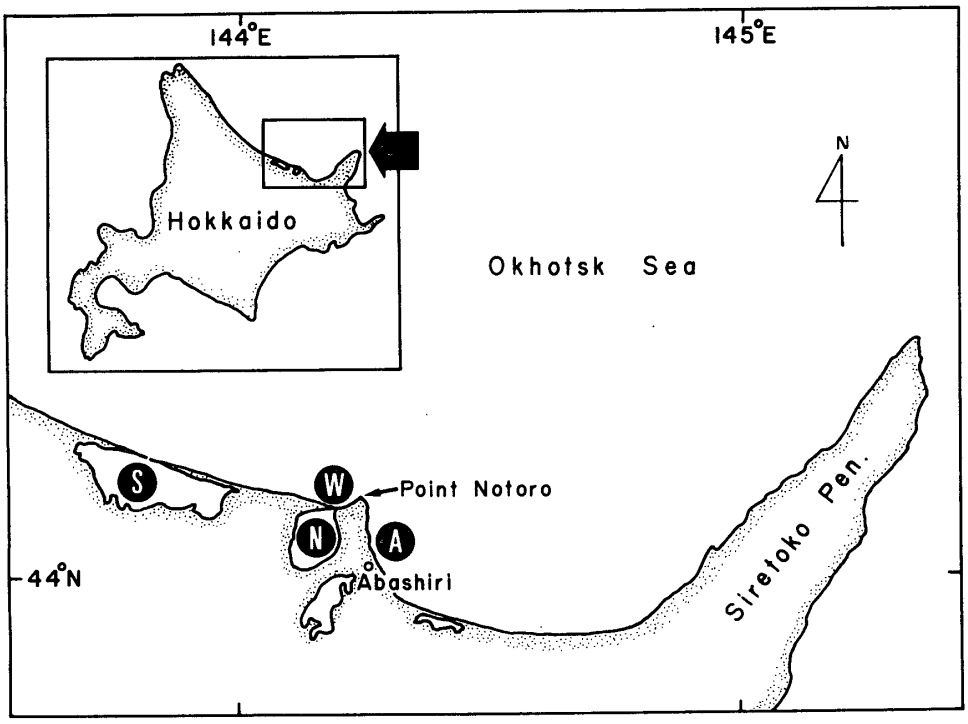

FIG. 1. Four areas where the specimens were collected: Abashiri Bay (Ⓐ), the west of Point Notoro (Ⓦ), Lake Saroma (Ⓢ) and Lake Notoro (Ⓝ).

To investigate the oocyte size and the nucleus diameter, thirty oocytes, which are representative of the main size group, from the ovarian tissue of each female specimen were measured monthly by a measure microscope (Fusoh Co., Japan). Only those oocytes sectioned through the nucleolus were measured.

The genital tubules on the preparation from each animal were randomly counted 5 times successively. They were calculated and converted into the number of genital tubules per mm^2.

The data on possible duration of sunshine (hr/day), sunshine duration (hr/day), global solar radiation (MJ/m^2/day) and drifting ice period (DIP) (days) were obtained from the Abashiri Meteorological Observatory.

Results

1. *Changes in Gonad Index and Oocyte Size*

As shown in Fig. 2, the gonad index in May 1983 was about 15. However, it dropped abruptly in June and July. It increased from 5 in September 1983 to 22 in May 1984, which showed the highest value over the entire period of the study. The monthly changes in the oocyte size and nucleus diameter also showed the same trend, but it is noticeable that the oocyte size in May 1983, which showed 60 μm, was larger than that in May 1984. There was no change in the values of oocyte size or nucleus diameter from July to September in 1983. On the other hand, no appreciable difference was found among the monthly changes of the three

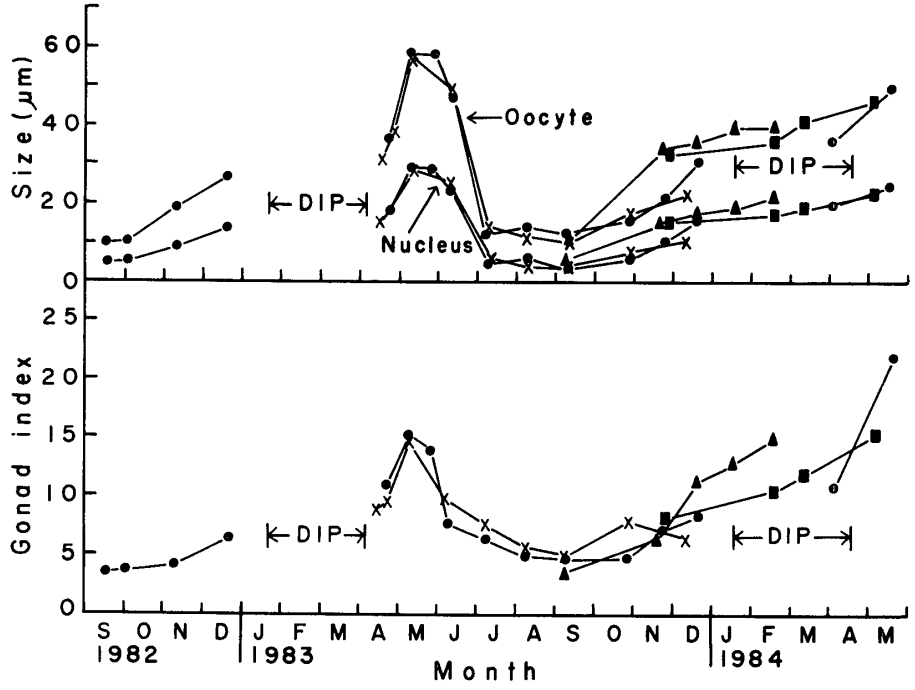

FIG. 2. Monthly changes in oocyte size and nucleus diameter and in gonad index in four areas of Abashiri Bay (—×—), the west of Point Notoro (—●—), Lake Saroma (—▲—) and Lake Notoro (—■—). DIP,, drifting ice period.

factors stated above between Abashiri Bay and the west of Point Notoro. However, it appeared that a significant difference existed between Abashiri waters and Lake Saroma. The oocyte size in Lake Saroma (40 μm) in February 1984 was smaller than that in Abashiri waters (60 μm) in May 1983, while the gonad indices in the two areas in both months had the same value of 15.

2. Changes in Number of Genital Tubules

The monthly variations in number of genital tubules per mm^2 (Fig. 3) are inverse to those of gonad index, oocyte size and nucleus diameter (Fig. 2). Moreover, there was no difference in the trends between Abashiri Bay and the west of Point Notoro. It is noteworthy that the number of genital tubules in May 1983 was more than that in May 1984.

3. Correlation between Oocyte Size and Nucleus Diameter

The relationship of growth between the oocyte and its nucleus is plotted in Fig. 4. By linear regression we showed the existence of a linear relation between the two parameters and it appeared that the nucleus diameter increased in proportion to the growth of the oocyte to which it belonged.

4. Identification of Gonadal Development Stages

Based on morphological features and the sizes of the germ cells and tissue cells around them, the gonadal phases could be classified into five successive

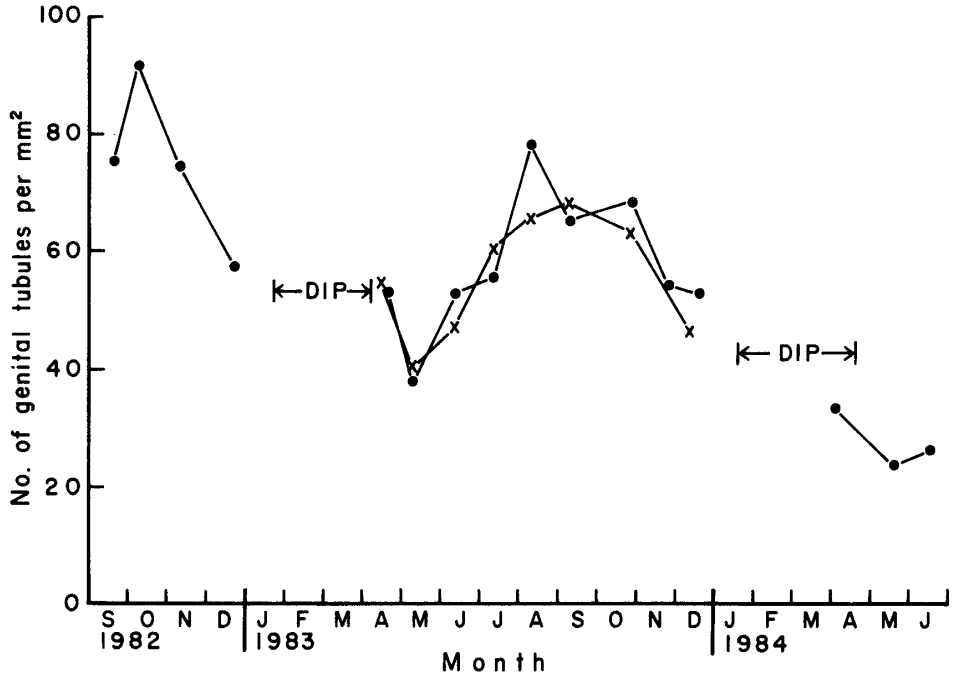

FIG. 3. Monthly changes in number of genital tubules per mm^2 in the preparation of gonad. Symbols as in Fig. 2.

stages. The criteria used in defining the categories are as follows.

1) Multiplicative stage

Early (Fig. 5-Mul) ; Oogonia propagate in the germinal epithelium and have round nuclei containing nucleoli in their centers. The nucleus and nucleolus are distinct in appearance, though the cytoplasm of oogonium is still very poor. Oogonium is about 10 μm in size and nucleus diameter (n.d.) is 7-10 μm. There are less than 10 oogonia in one tubule. The number of spermatogonia is less than that in Mu2. Connective tissues develop well among the tubules.

Late (Fig. 5-Mu2) ; Histological details are more or less the same as in Mu1, but there are more than 10 oogonia per tubule. Size of oogonium is about 10 μm and n.d. is 8-10 μm. Spermatogonia are seen in a row along the germinal epithelium. Others are the same as in Mu1.

2) Growing stage

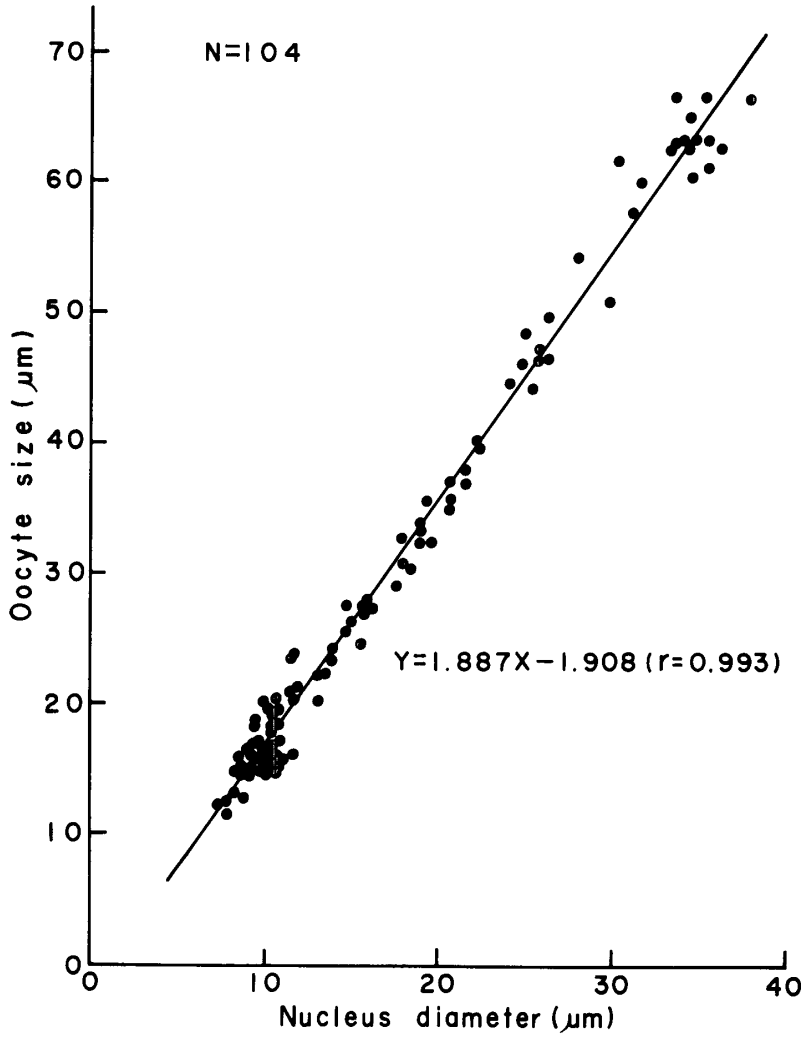

FIG. 4. Relationship between nucleus diameter and oocyte size in female gonads of the scallops in sowing cultures in Abashiri waters.

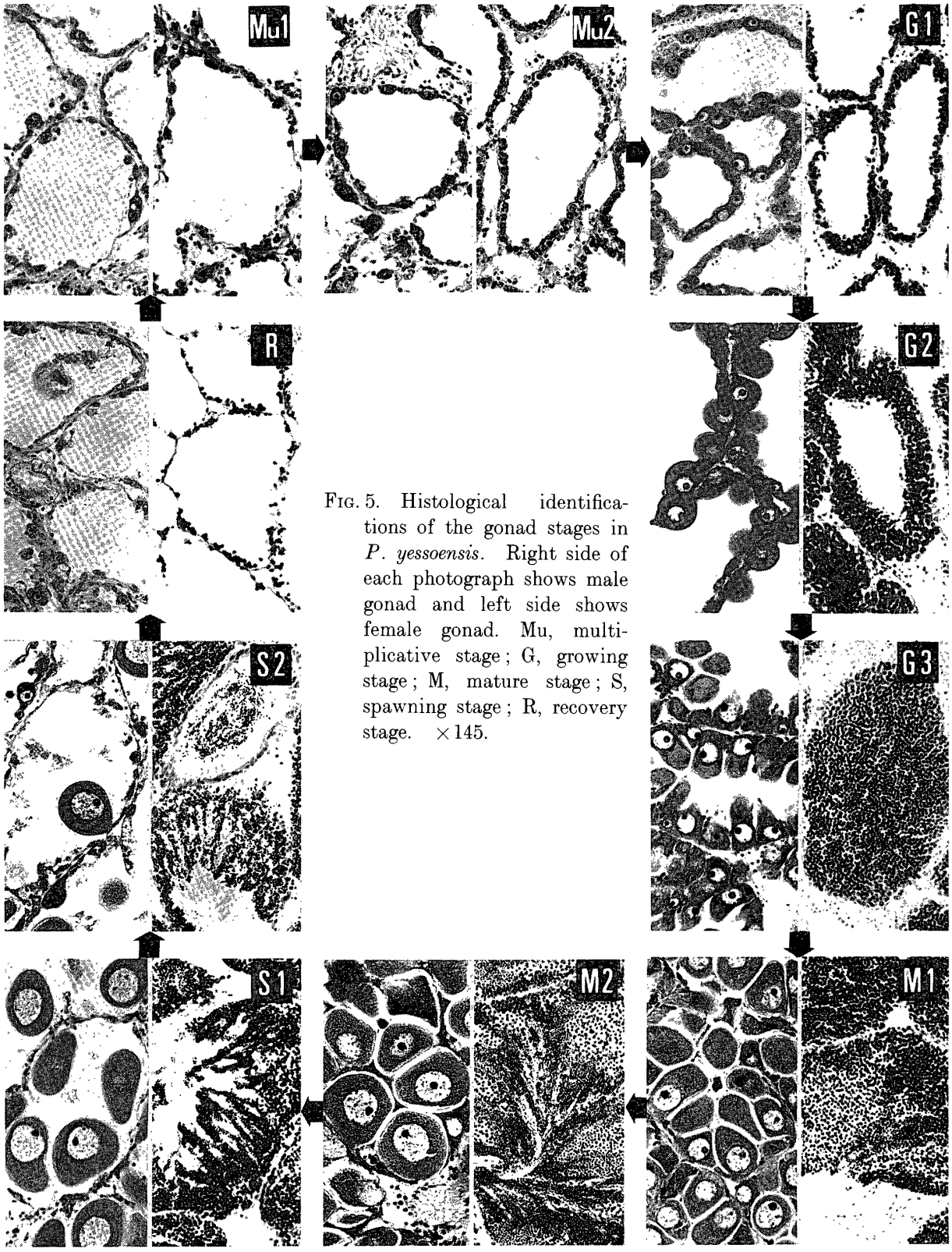

FIG. 5. Histological identifications of the gonad stages in *P. yessoensis*. Right side of each photograph shows male gonad and left side shows female gonad. Mu, multiplicative stage; G, growing stage; M, mature stage; S, spawning stage; R, recovery stage. ×145.

Early (Fig. 5-G1); Lumen is still empty. Oocytes measure 17-31 μm, and have developed cytoplasm. The nucleus has a diameter of 9-18 μm, and is still rather larger than the cytoplasm. Spermatocytes form the duplicative arrangement on the germinal epithelium and occupy about 20% of the lumen volume.

Middle (Fig. 5-G2); Oocytes increase the volume of their cytoplasm more than those in G1 and grow on towards the center of the lumen. They occupy about 40% of the lumen volume. Oocytes measure 29-45 μm in size and n.d. is 17-26 μm. Spermatocytes occupy about 40 or 50% of the lumen volume. It is often observed that the lumen is filled with spermatocytes.

Late (Fig. 5-G3); Most of the oocytes contain much yolk materials and arrange around the center of the lumen. The oocytes measure 35-51 μm, fill up the tubule and n.d. is 19-27 μm. The testicular tubule increases its volume and contains stratified layers composed respectively of spermatogonia, spermatocytes and spermatids on the germinal epithelium. The lumen is filled with these cells.

3) Mature stage

Early (Fig. 5-M1); The majority of oocytes are gradually becoming round or oval. Oocyte size is 40-55 μm and n.d. is 22-31 μm. A few spermatids formed by meiosis begin to undergo transformation into spermatozoa in the center of the lumen.

Late (Fig. 5-M2); Oocytes grown fully are surrounded by gelatinous membrane and their cytoplasms contain a large number of yolk granules. The ovarian tubule reaches to the largest volume all over the stages. Oocytes measure 54-71 μm and n.d. is 28-38 μm. In male gonads most of the lumina are filled with countless spermatozoa. Their heads orient to the germinal epithelium, and their tails to the center of the lumen. These numerous spermatozoa form a flowing structure. A small number of spermatocytes and spermatids are observed near the germinal epithelium in the testicular tubule.

4) Spawning stage

Early (Fig. 5-S1); The lumen becomes considerably empty, since about 30% of oocytes in a tubule are discharged. Oocyte size is the same as in M2. Testicular tubule loses the flowing structure formed by countless spermatozoa in its lumen, as a fairly large number of spermatozoa are discharged into the surrounding environment.

Late (Fig. 5-S2); Fully grown oocytes are entirely discharged and the shrunken tubules have a few ripe undischarged oocytes as well as young oocytes. A few remaining spermatozoa are scattered in the shrunken lumen. Spermiducts with the spermatozoa are often observed.

5) Recovery stage (Fig. 5-R); After the spawning, the undischarged oocytes in the lumen undergo cytolysis and each ovarian tubule is contracted. Testicular tubules are also contracted and empty. Connective tissues develop among the tubules.

TABLE 1. *Comparison of Gonadal Phases in Abashiri Waters with Those in Nearby Lakes*

Date	Area	No. of sample	Mu1	Mu2	G1	G2	G3	M1	M2	S1	S2	R
Sep. 9, 1983	A	8	●									•
〃	W	8	●	●								•
〃	S	7	•	●	•						•	
Oct. 28	A	8	●	●								
〃	W	8		•	●							
Nov. 24	W	10		●	●							
Nov. 21	S	10			•	●						
Nov. 24	N	10			●	●	•					
Dec. 12	A	10			●							
Dec. 19	W	8			●	●						
〃	S	8					●	●				
Jan. 17, 1984	S	10					●	●				
Feb. 17	S	10					•	●	●			
Feb. 16	N	10			•	●	•	•				

A, Abashiri Bay; W, the west of Point Notoro; S, Lake Saroma; N, Lake Notoro; Mu, multiplicative stage; G, growing stage; M, mature stage; S, spawning stage; R, recovery stage; •, 0-20%; ●, 21-40%; ●, 41-60%; ●, 61-80%; ●, 81-100%.

5. *Seasonal Changes in Gonadal Phases*

According to the criteria of gonadal stages together with gonad index, a comparison of gonadal phases in the two areas of Abashiri waters with those in nearby lakes of Saroma and Notoro was performed as shown in Table 1. In September, the gonad of the scallop in hanging cultures in Lake Saroma reached the growing stage, while more than 80% of the scallop gonads in sowing cultures in Abashiri waters were in the multiplicative stage. In addition, the gonad began to mature in Lake Saroma in December, but in Abashiri waters it remained in the growing stage. The comparison between specimens from Abashiri Bay and from the west of Point Notoro indicated that the latter were somewhat more rapid in the development of gonad. However, in general the seasonal changes in gonadal phases in the two areas were almost identical to each other. In Lake Notoro, the gonad developed to the same degree as in Lake Saroma in November, but that growth slowed greatly during winter.

Since there are no significant differences in gonad indices, in sizes of oocytes and in number of genital tubules between the scallops in Abashiri Bay and those in the west of Point Notoro, the results of the two areas were combined as shown in Table 2. Moreover, since males and females shown no appreciable difference in

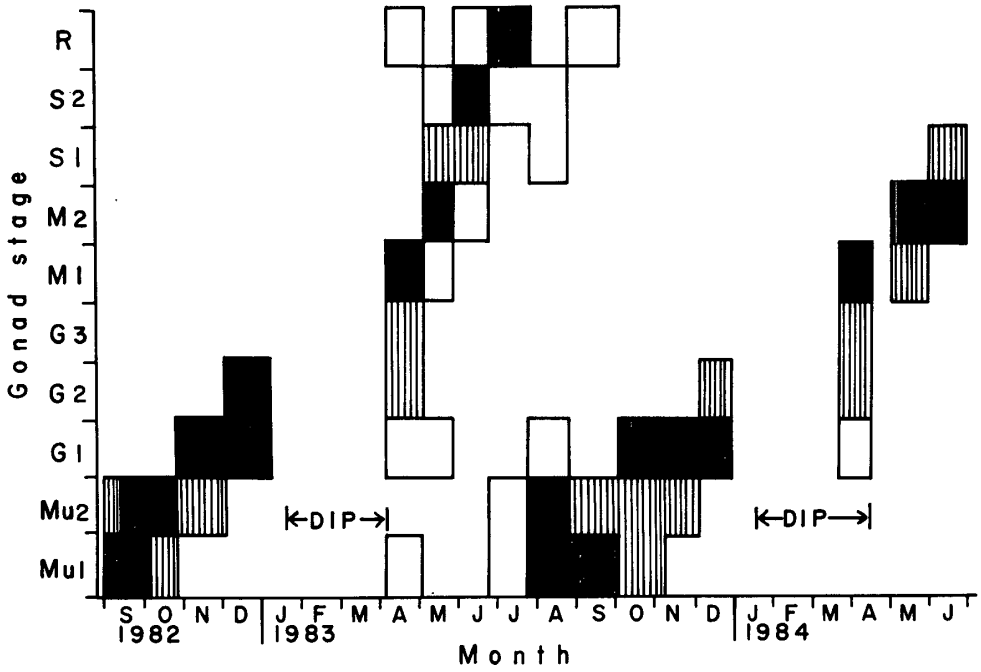

FIG. 6. Monthly composition of gonadal phases in the scallops in sowing cultures from September 1982 to June 1984. ■, the highest percentage (mode); ▥, above 20%; □, below 20%; Mu, multiplicative stage; G, growing stage; M, mature stage; S, spawning stage; R, recovery stage; DIP, drifting ice period.

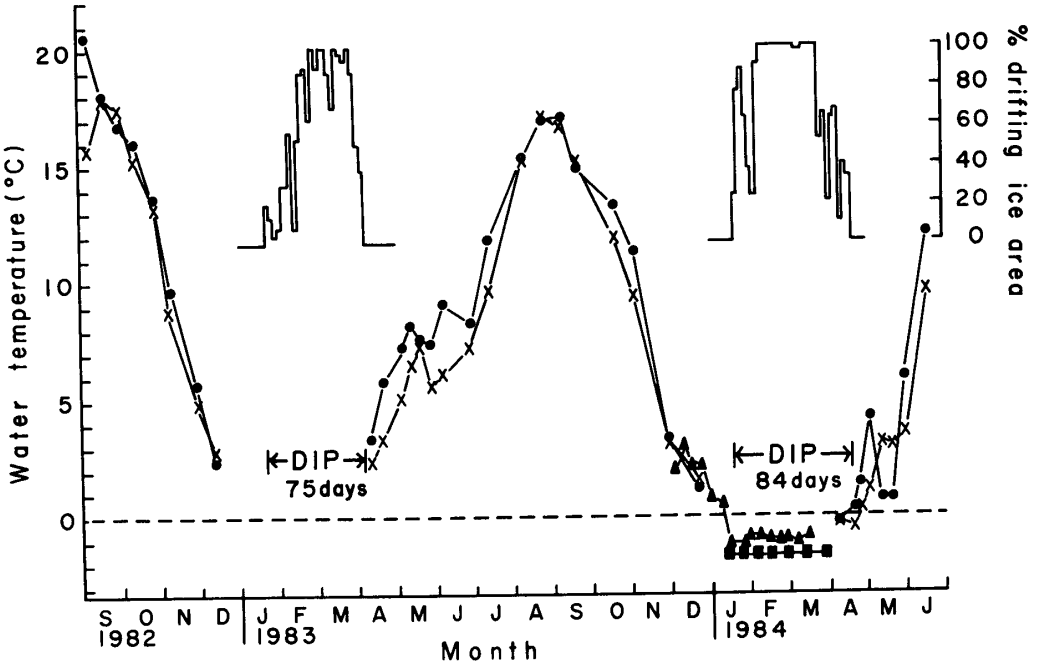

FIG. 7. Fluctuations of water temperatures at 30 m depth in Abashiri Bay (—×—), 30 m depth in the west of Point Notoro (—●—), 2 m depth in Lake Saroma (—▲—) and 6 m depth in Lake Notoro (—■—), and of the percentage of drifting ice area observed daily during the drifting ice period (DIP) in Abashiri Bay. The percentage of drifting ice area represents the ratio of drifting ice area to the sea surfaces of the limited area in Abashiri Bay.

TABLE 2. *Monthly Distributions of the Various Stages of Gonadal Development and*

Date of sampling	W.T. (°C)	No. of sample	Male						
			Mu1	Mu2	G1	G2	G3	M1	M2
Sep. 16, 1982	18	10	3						
Oct. 3	16	10	4	2					
Nov. 11	9	10		2	3				
Dec. 21	2	10			5				
Apr. 19, 1983	5	25			1	4	2	4	
May 15	7	24			1			1	5
Jun. 9	8	16							
Jul. 8	11	16							
Aug. 9	15	16	3	2					
Sep. 9	17	16	3	1					
Oct. 28	10	16	4	3	1				
Nov. 24	3	10		2	2				
Dec. 15	2	18			12	1			
Apr. 4, 1984	−0	10			1	1	2	3	
May 18	2	10							5
Jun. 15	12	11							1
Main period in each stage			Aug.-Nov.		Oct.-May			Apr.-Jun.	

W.T., water temperature; Mu, multiplicative; G, growing; M, mature; S, spawning; R, recovery

gonadal phase as indicated in Table 2, pooled data for the two sexes are given in one figure (Fig. 6), indicating the percentages in each stage. From Fig. 6, it was deduced that the scallops in sowing cultures in Abashiri waters have a progressive reproductive cycle as follows: the multiplicative stage from July to November, the growing stage from October to April, the mature stage from March to June, the spawning stage from May to July and the recovery stage in June and July.

We have investigated 228 scallop gonads in sowing cultures during this study, but found no abnormal gonad, such as overripe gonad, similar to those observed by Mori and Osanai (1) in hanging cultured scallops.

Discussion

1. *Relations between Environmental Factors and Reproduction*

It is thought that gonadal development and maturation in the scallops from Abashiri waters and nearby lakes of Saroma and Notoro occur from October to May. This period contains the DIP when sea water temperature is below freezing (Fig. 7). Fig. 8 shows very low solar radiation and very short sunshine duration during DIP. The sea surface of Abashiri waters is covered with thick ice during this period. Therefore, the relative intensity of solar radiation beneath the

Spawning in the Scallop from Sowing Cultures in Abashiri Waters from 1982 to 1984

			Female									
S1	S2	R	Mu1	Mu2	G1	G2	G3	M1	M2	S1	S2	R
			2	5								
				4								
					5							
						5						
		1	1			1	6	5				
7									6	3	1	
3	3	2							3	1	3	1
	1	10	1	1								3
2	1		3	4	1							
		2	7	3								
				2	6							
				1	5							
					2	3						
							1	1	1			
								2	3			
3									5	2		
May-Aug.		Jun.-Jul.	Jul.-Nov.		Oct.-Apr.			Apr.-Jun.		May-Jun.		Jun.-Jul.

drifting ice is very low as below 1% of that above the drifting ice (4). In Lake Saroma phytoplankton in the water column beneath the ice are very poor in volume, because of the low light intensity (5). The onset of gonadal development and maturation in bivalve molluscs are closely related with the rising water temperature (6-10), the abundance of plankton (7-9) and the long duration of daily sunshine (9, 11). Gonadal growth and cytoplasmic growth of oocytes are induced in a scallop, *Aequipecten irradians*, exposed to 15°C with food, but only oogonia develop at 5°C (12). However, it is noticeable that the scallop in our study continued its gonadal development even during DIP, in spite of extremely low water temperature and insufficient food supply. Similarly, Sundet and Lee (13) reported that the production of precholion oocytes in the gonad of the Iceland scallop, *Chlamys islandica*, collected from Lat. 70°N (14), continued throughout the autumn and winter, reaching a peak in February. However, since the researchers did not deal with the sea water temperature, it is difficult to compare their results with those in our study. As far as we know, no reference has been made to the scallop gonad in the habitat having DIP which shows sea water temperature below the freezing point.

As indicated in Fig. 7, the volume and duration of drifting ice in Abashiri

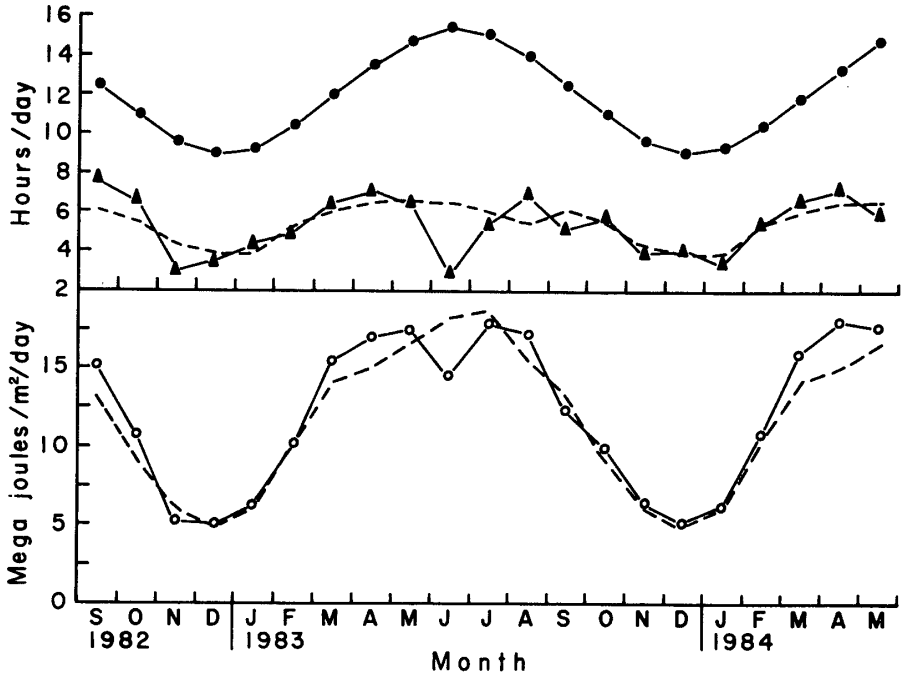

FIG. 8. Monthly variations of possible duration of sunshine (hr/day) (—●—), sunshine duration (hr/day) (—▲—) and global solar radiation (MJ/m²/day) (—○—) in Abashiri waters during the investigation. Dotted lines indicate the averages for the last 30 years. These data were obtained from the Abashiri Meteorological Observatory.

waters varies according to the year, as does the water temperature. The water temperature and gonad index for April and May 1984 were −0.5-4°C and 10-22 respectively, while those during the same months in 1983 were 3-8°C and 10-15 respectively. On the other hand, the oocyte size in May 1983 (60 μm) was larger than that of 1984 (50 μm), suggesting that gonad maturation and spawning depend upon environmental conditions of each year.

2. *Variations of Gonadal Conditions*

In September 1983 there was no difference for gonad indices between Abashiri waters and Lake Saroma. Thereafter, the values showed differences with successive month. In February 1984 the value in Lake Saroma was 15, the same as that in Abashiri waters in May 1983 which showed the highest value through the year. In the cytological observation, however, the oocyte size was 40 μm in February 1984 in Lake Saroma and 60 μm in May 1983 in Abashiri waters. These comparisons demonstrated that in spite of having the same gondad index, the scallops in Abashiri waters were in the mature or spawning stages, while those in Lake Saroma were in the growing stage. Hence, it can not be said that gonad index is an absolute device in determining spawning season or reproductive cycle and also in forecasting the spat collection.

The number of genital tubules per mm² in gonad tissue in this study exhibit-

ed a minimum in May and a maximum in August. This trend suggests that the genital tubules periodically increase and decrease in volume. It is expected that this phenomenon in the genital tubules may play a role in discharging the gametes into the surrounding environment.

3. *Reproductive Periodicity*

The scallop gonad in sowing cultures in this study proliferated its germ cells from July to November and developed them from October to April including the DIP. Maturation of gonads began to appear in March and continued to June. Spawning began in May and ended in July. The recovery of the gonad occurred in June and July. The reproductive cycle described above is comparable with that by Maru (9) who studied the scallop in hanging cultures in Lake Saroma as shown in Table 3. In the growing stage the gonadal development in our study was two or three months later in comparison with his results. In other stages our

TABLE 3. *Comparison of the Reproductive Cycles of the Scallops by Sowing Culture in Abashiri Waters and by Hanging Culture in Lake Saroma*

Sowing culture (Present authors)	Gonad stage	Hanging culture (Maru, 1976)
Jul.-Nov.	Multiplicative 1 and 2	Jul.-Oct. (Resting)
Oct.-Apr.	Growing 1 and 2	Nov.-Jan. (Early growing)
Mar.-May	Growing 3 and Mature 1	Feb.-Mar. (Late growing)
Mar.-Jun.	Mature 2	Apr.-Jun. (Maturing)
May-Jun.	Spawning 1	May-Jun. (Breeding)
May-Jul.	Spawning 2 and Recovery	Jun. (Spent)

( ), gonad stage presented by Maru (1976).

TABLE 4. *Spawning Periods of Various Scallop Species in Different Localities*

Species	Locality (latitude)	Spawning period	Reference
Patinopecten yessoensis	Toni Bay, Japan (39°N)	Apr.-May	(15)
	Funka Bay, Japan (42°N)	Apr.-Jun.	(16)
	Lake Saroma, Japan (44°N)	May-Jun.	(9)
	Abashiri waters, Japan (44°N)	May-Jul.	Present study
Patinopecten caurinus	Gulf of Alaska, U.S.A. (60°N)	Jun.-mid Jul.	(17)
Placopecten magellanicus	Newfoundland, Canada (47°N)	Aug.-Sep.	(18)
	Newfoundland, Canada (49°N)	Sep.-Oct.	(19)
Chlamys tigerina	Isle of Man, U.K. (54°N)	Jun.	(20)
Chlamys varia	Isle of Man, U.K. (54°N)	Jun., Sep.-Oct.	(20)
Chlamys striata	Isle of Man, U.K. (54°N)	Aug.	(20)
Chlamys islandica	Tromsø, Norway (70°N)	Late Jun.-early Jul.	(13)

results more or less agreed with his. In this respect, it seems likely that the scallops in Abashiri waters and in Lake Saroma are somewhat different in the period of gonadal growth, but consistent in the periods of their maturation and spawning. The simultaneity in the spawning might be dependent on the reproductive strategy in the scallops in the same latitude having similar environments.

The reproductive cycles of the scallop, *P. yessoensis* and related species vary with localities. The varieties of their spawning periods are indicated in Table 4 (9, 13, 15-20). These variations in reproductive periods might be related to the geographical differences in sea water temperature (10, 21) and time of abundant food production (21) and to the differences in scallop species.

Acknowledgements

Thanks are due to the staff of the Abashiri Scallop Research Laboratory, Hokkaido, for collection of the scallops. This study was supported in part by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science and Culture.

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