Studies on Sharks–XII Monthly Change of the Gonad Index in Male *Mustelus manazo* and *M. griseus**

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Genus *Mustelus* occurring in Japan consists of two species, *M. manazo* Bleeker and *M. griseus* Pietschmann (see Teshima and Koga, 1973). Since the external characters of both species are similar to each other, it is not easy to distinguish them. The only point of difference in external characters is the relative length of the upper and lower labial folds. However, it has become clear that there is a distinctive difference in reproduction between the two species. In *M. griseus*, the placenta is established in the middle stage of gestation and the embryo is nourished by mother after establishment of the placenta. In *M. manazo*, the placenta is not established at all throughout the whole stage of gestation and the embryo is nourished by yolk stored in its yolk sac. As described here, sharks in genus *Mustelus* have a interesting reproductive system. The present study was directed a describing the monthly change of the gonad index and the composition of spermatogenic cells in male *M. manazo* and *M. griseus*.

Materials and methods

The male specimens of *Mustelus manazo* were collected monthly during the period from July, 1975 to May, 1976, and those of *M. griseus* from May, 1974 to May, 1975 at the Shimonoseki central fish market, Shimonoseki, Japan. The number of specimens investigated is 150 in *M. manazo* and 110 in *M. griseus* (Table 1). These sharks were caught mainly by bull trawlers in the East China Sea.

Testes taken were preserved in 10% formalin and their weight was recorded. Gonad index (GI) was calculated from the following formula, $GI = W/TL^3 \times 10^6$, W: testis weight (g), TL: total length (cm). In order to examine the monthly change of spermatogenic cells developing in the testis, histological sections were made of all the testes collected. Testes were imbedded by paraffin, cut at 5~8 μ thickness and stained with Hanzen's haematoxylin and eosin.

Results

Monthly gonad index (Fig. 1)

The monthly gonad index shows the same trend in both *Mustelus manazo* and *M. griseus*. The value of the gonad index reaches the minimum in June to July and ascends after this.

Table 1. Total length and the number of male *Mustelus manazo* and *M. griseus* specimens used in the present investigation.

Mustelus manazo		*Mustelus griseus*	
Date collected	No. of specimens (Total length in mm)	Date collected	No. of specimens (Total length in mm)
1975 July	14 (507~693)	1974 May	7 (732~813)
Aug.	20 (544~677)	June	10 (615~772)
Sept.	21 (496~671)	July	11 (638~790)
Oct.	16 (652~825)	Aug.	18 (568~783)
Nov.	11 (646~970)	Sept.	6 (726~758)
Dec.	7 (652~736)	Oct.	7 (660~758)
1976 Jan.	4 (710~753)	Nov.	10 (710~827)
Feb.	10 (477~762)	1975 Jan.	6 (791~909)
Mar.	12 (450~1010)	Feb.	11 (752~888)
Apr.	18 (638~728)	Mar.	2 (820~879)
May	17 (683~832)	Apr.	5 (720~872)
		May	17 (712~814)
Total	150 (450~1010)		110 (615~909)

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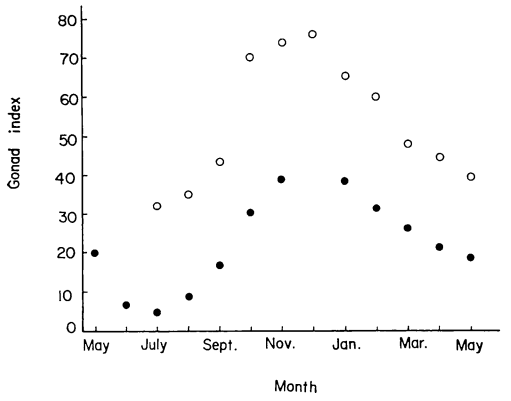

Fig. 1. Monthly gonad index in *Mustelus manazo* (open circle) and *M. griseus* (closed circle).

The value reaches the maximum in November to December and descends after this. The value of the gonad index varies between 30 and 80 in *M. manazo* and between 5 and 40 in *M. griseus*.

Monthly change of spermatogenic cells developing in the testis (Fig. 2)

In both *Mustelus manazo* and *M. griseus* spermatogonia, spermatocytes, spermatids and spermatozoa are found in the testes of all the specimens throughout the year. However, the composition of these spermatogenic cells is different by month. In both species the seminiferous tubules containing the spermatogonia exist in the ventral side of the testis. The spermatogenic cells contained in the tubules change from spermatogonia to spermatocytes and spermatids as they move towards the dorsal side; the seminiferous tubules containing the complete spermatozoa exist in the dorsal side of the testis. Within one seminiferous tubule, most spermatogenic cells are in the same developmental stage.

Figure 2 indicates the monthly change of the occupying ratio of seminiferous tubules containing the spermatogenic cells in the various developmental stages in the transverse section of the middle portion of the testis. In both species a remarkable change is found in the occupying ratio of spermatocytes and spermatozoa throughout the year. Spermatogonia and spermatids show no significant change in their occupying ratio. When many spermatocytes are observed, the number of spermatozoa is small. On the other hand, when the number of spermatocytes developing is small, many spermatozoa are formed. In both species the occupying ratio of seminiferous tubules containing the spermatozoa is extreamly low in July and August. In September, as the month advances, the spermatogenesis becomes vigorous. In December the number of seminiferous tubules containing spermatocytes, spermatids, and spermatozoa becomes about equal. The occupying ratio of seminiferous tubules containing spermatozoa reaches the maximum by March in *Mustelus manazo* and May in *M. griseus*. In *M. manazo*, the number of seminiferous tubules containing the spermatozoa decreases during the period from April to June and the occupying ratio reaches the minimum in July and August. In *M. griseus*, it decreases rapidly in June and reaches the minimum in July and August.

Testes reach their yearly minimum in weight, length, and diameter in July and August, and increase in their size as the month advances after this. They reach their yearly maximum in December, and decrease after this.

Condition in the seminal vesicle

Both *Mustelus manazo* and *M. griseus* have the long spermiducts and seminal vesicles. The seminal vesicle is formed by an expansion of the anterior end of the spermiduct. Spermatozoa produced in the testis are accumulated in the seminal vesicle after passing through the spermiduct.

No spermatozoa are found in the spermiducts and the seminal vesicles of specimens in September and October. A small quantity of spermatozoa are found in those seminal vesicles examined in November and December. Spermatozoa in the seminal vesicle are accumulated after December, and the seminal vesicle becomes fully filled with spermatozoa by June. However, the seminal vesicles and spermiducts of specimens in July and August are either filled with spermatozoa or quite empty.

Discussion

Concerning the marine teleosts, Yamamoto (1953), working with *Liopsetta obscura* (Herzenstein), and Mizue (1957, 1958, 1959, 1961), with *Sebastiscus marmoratus* (Cuvier et Valenciennes), *Sebastes inermis* Cuvier et Valenciennes, and *Ditrema temmincki* Bleeker, have described in detail the monthly changes of the gonad indices of these species. According to Yamamoto

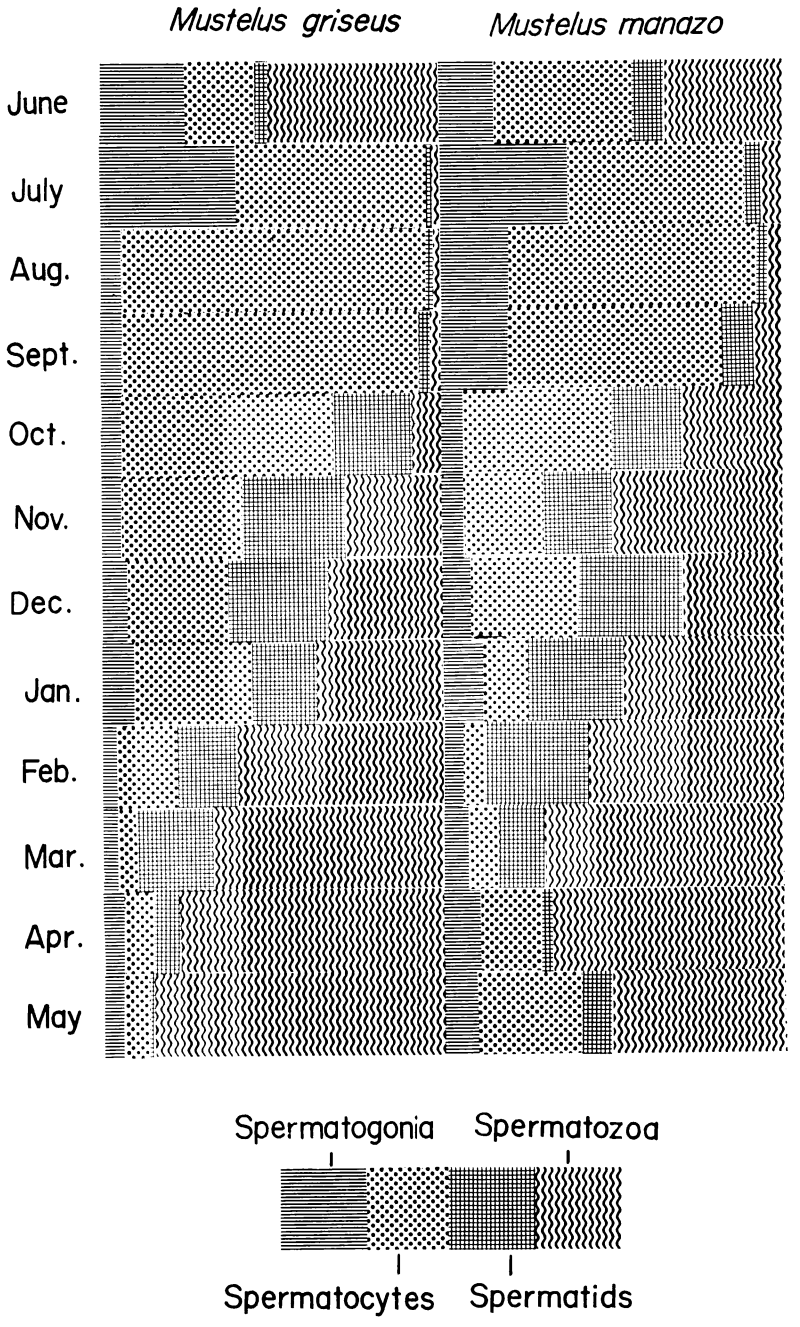

Fig. 2. Monthly composition of spermatogenic cells in the testis of *Mustelus manazo* and *M. griseus*.

and Mizue, the discharge of spermatozoa in the fishes occurs when the value of the gonad index starts descending or is descending from the maximum.

In both *Mustelus manazo* and *M. griseus*, a time lag is found between the peak in the gonad index and the maximum in the occupying ratio of the seminiferous tubules containing the spermatozoa. The occupying ratio reaches the maximum four to five months later than the

peak of gonad index. However, this does not indicate that the testis is most active when the occupying ratio of seminiferous tubules containing the spermatozoa reaches the maximum. Although the occupying ratio of spermatozoa increases during the period from December to April or May, the space occupied by seminiferous tubules containing the spermatozoa in the testis is almost the same during this period due to reduction of testis weight, length and diameter. During the period from December to around April or May, when the value of the gonad index is descending, spermatozoa are thus produced vigorously and accumulated in the seminal vesicle.

The lag between peak in gonad index and spermatozoa discharge in *Mustelus manazo* and *M. griseus* (as compared to the teleosts described by Yamamoto and Mizue, in which a peak in gonad index and spermatozoa discharge occur at the same time) is due to the structural differences in the reproductive systems. In both *M. manazo* and *M. griseus*, the spermatozoa produced are sent out of the testis when the value of the gonad index is descending. Due to the fact that both *M. manazo* and *M. griseus* have very long spermiducts and expanded seminal vesicles, spermatozoa sent out of the testis are not discharged out of the body immediately, but are accumulated in the seminal vesicle.

In the gonad index graph, when the value reaches the maximum, a small quantity of spermatozoa are found in the seminal vesicle. Conversely the seminal vesicle becomes filled with spermatozoa, as the value reaches the minimum.

Spermatozoa are usually discharged when the testis activity is greatest in the teleosts (Mizue, 1957, 1958, 1959), but the discharge of spermatozoa in both *Mustelus manazo* and *M. griseus* takes place when the testis activity is at its lowest.

No spermatozoa are found in the spermiducts and the seminal vesicles in September and October. Additional to this, the seminal vesicle becomes fully filled with spermatozoa by June. These facts suggest that the spermatic fluid filling the seminal vesicle is discharged out of the body in June to August, i. e., mating in both species takes place in this period. In the gonad index graph, mating occurs when the value is minimum.

In both *Mustelus manazo* and *M. griseus* females, ovulation occurs in June and July (Teshima and Koga, 1973; Teshima et al., 1974). The ovulating season agrees with the mating season estimated by the monthly change of the gonad index and the condition of the seminal vesicle in *M. manazo* and *M. griseus*. Ovulation, mating, and fertilization in Japanese *Mustelus* species thus take place during the period from June to August.

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サメ類の研究—XII. ホシザメおよびシロザメ雄の生殖腺指数の月別変化

手島　和之

ホシザメ *Mustelus manazo* およびシロザメ *M. griseus* 雄の生殖腺指数と組織学的方法による精巣中の各精細胞の出現率の月別変化を調査した．両種とも生殖腺指数は6月より7月にかけて最小になり，11月から12月にかけて最大になる．精子は両種とも生殖腺指数の値が最小になる時放出される．すなわち，6月より8月の間が両種の交尾時期であると考えられる．両種ともに，精子形成は周年休むことなく行なわれる．このため，採集したすべての標本の精巣中に精原細胞，精母細胞，精子細胞および精子の各精細胞が観察される．しかしながら，各精細胞の出現率は月によって異なっている．精母細胞が多く出現する時は精子は少なく，反対に，精母細胞の出現が少い時は精子は多数存在する．

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