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Sexual Maturity of *Raja koreana* (Elasmobranchii, Rajoidei) from Korea

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Sexual maturity of Korean skate, *Raja koreana*, was investigated based on 89 specimens caught between September 2002 and December 2003 in the southwestern sea of Korea. Clasper development was slow between 35 cm and 52.5 cm TL, increased abruptly between 59.5 cm and 71 cm TL, and thereafter decreased, showing an overall sigmoidal growth pattern. Unlike other skates and sharks, Korean skate males were already histologically mature before the claspers lengthened. Although the egg capsules were found in May and November in Korean skate females, histological findings indicated that spawning occurred throughout the year except summer in females but all the time in males.

Key words : sexual maturity, clasper, *Raja koreana*

Introduction

The Korean skate, *Raja koreana* (Korean name : Koryo-hongeo) was originally described based on one specimen from the southwestern sea of Korea (Jeong and Nakabo, 1997). The species is distributed in Jeju Strait, Korea (Jeong and Nakabo, 1997) and among Tsushima and Goto Islands, Japan (Tokimura *et al*., 1998). But, Jeong (1999) considered the occurrence from Japan as a chance, current-assisted, dispersal event.

Traditionally, the Korean skates, of which mottled skate (*Raja pulchra*) and spiny rasp skate (*Okamejei kenojei*) have been regarded as the most favorite species. However, since their catches have been rapidly decreased in recent years (NFRDI, 2005), human consumption of Korean skate has shown a tendency of increase instead of the two commercial skates (mottled skate and spiny rasp skate). The resources condition of the Korean skate may be in danger if its exploitation would be in excess of sustainable yield.

Elasmobranch fishes have life history strategies that rely on greater offspring survival and longevity after maturation (Frisk *et al*., 2001). A number of studies on the reproduction and population dynamics of skates have been conducted, e.g., growth and population dynamics of three *Raja* species (Ryland and Ajayi, 1984), growth rate of *Raja clavata* (Brander and Palmer, 1985), reproduction of *Raja pulchra* (Yeon *et al*., 1997), age and maturity of *Leucoraja ocellata* (Sulikowski *et al*., 2005). Concerning the Korean skate, however, there have been no studies on biological characteristics such as maturity, age and growth.

The purpose of the present study was to present the sexual maturity of *R. koreana* based on histological observations, in addition to development of clasper in males.

Materials and Methods

Korean skate (Fig. 1) was caught by long line

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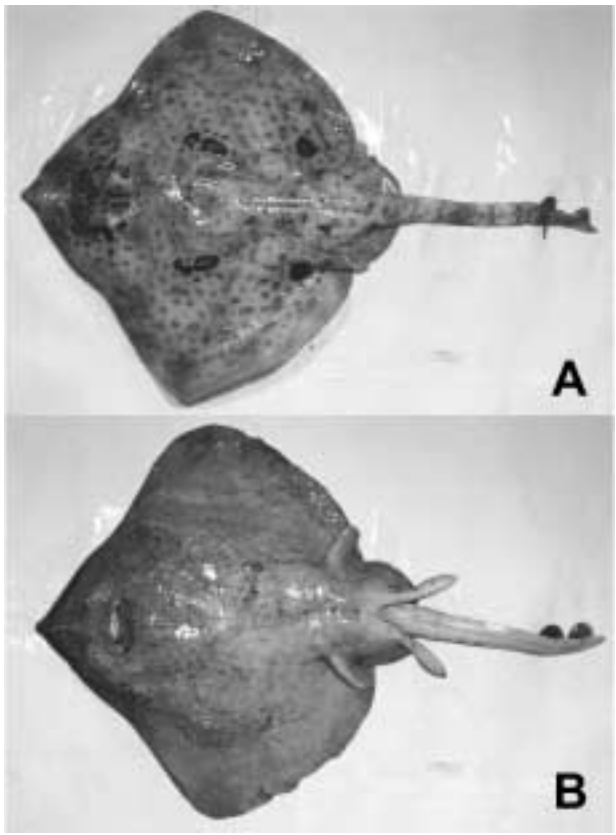

Fig. 1. *Raja koreana* from Korea. A: Dorsal view of female, B: ventral view of male.

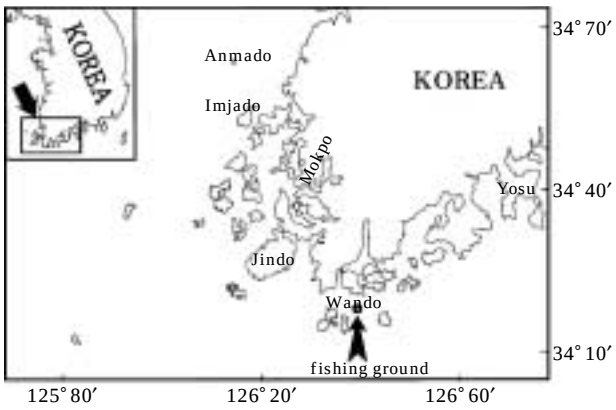

Fig. 2. Map showing the sampling area of *Raja koreana.*

in the southwestern sea of Korea (approx. 34° ~ 34° 20′N, 126° ~ 127° E, depth 30 ~ 70 m, see Fig. 2). Specimens were collected every month between September 2002 and December 2003 (Table 1), numbering 89 in total. Clasper growth was estimated by non-linear regression in the

Table 1. Sampling data of *Raja koreana* in the present study

	Date	Number of specimens		Total length (cm)	
		Female	Male	Female	Male
2002	September	–	1	–	73.0
	October	–	1	–	74.2
	November	5	5	61.0 ~ 75.0	68.2 ~ 83.9
	December	–	1	–	77.3
2003	January	1	2	67.0	72.2 ~ 74.0
	February	2	4	65.5 ~ 68.5	63.4 ~ 74.8
	March	4	6	41.4 ~ 75.3	43.9 ~ 71.0
	April	8	0	44.5 ~ 62.3	–
	May	3	4	56.0 ~ 74.7	42.2 ~ 73.7
	June	3	7	66.5 ~ 76.7	52.1 ~ 83.2
	July	2	1	58.5 ~ 79.8	65.2
	August	10	3	30.0 ~ 59.2	35.0 ~ 60.0
	September	–	–	–	–
	October	3	–	64.2 ~ 82.0	–
	November	2	3	50.0 ~ 55.0	67.2 ~ 73.9
	December	3	4	46.3 ~ 57.6	47.0 ~ 72.5
	Total	47	42	30.0 ~ 82.0	35.0 ~ 83.9

SigmaPlot (ver. 7.0), applying the following sigmoidal growth equation, $CL = a \cdot [1 + \exp\{-(TL - b) \cdot c^{-1}\}]$, where a, b and c are parameters. Gonadal tissues from 89 specimens were examined histologically. A portion of testis and ovary was fixed in Bouin's fluid, embedded in paraffin wax, sectioned at 3 ~ 5 μm and stained with Mayer's hematoxylin, followed by 0.5% eosin counterstain. The classification of sexual maturity was based on Ishiyama and Ishihara (1977), Hibiya (1982), Yasutake and Wales (1983) and Yeon *et al.* (1997).

Results

Male reproductive organ

Male reproductive organs comprised testis and claspers. The testis developed from the germinal epithelia and a seminiferous tubule was apparent in a specimen of 63.3 cm TL (November, 2002) (Fig. 3A). Spermatogonia (size ca. 25 μm) had a well stained nucleus (hematoxylin stain) and primary spermatocytes and spermatids appeared simultaneously in the testicular lobule in a specimen of 67.0 cm TL (January, 2003) (Fig. 3B). Primary spermatocytes, second spermatocytes and spermatids were clearly apparent in specimens of 63.3 ~ 81.0 cm TL, regardless of season

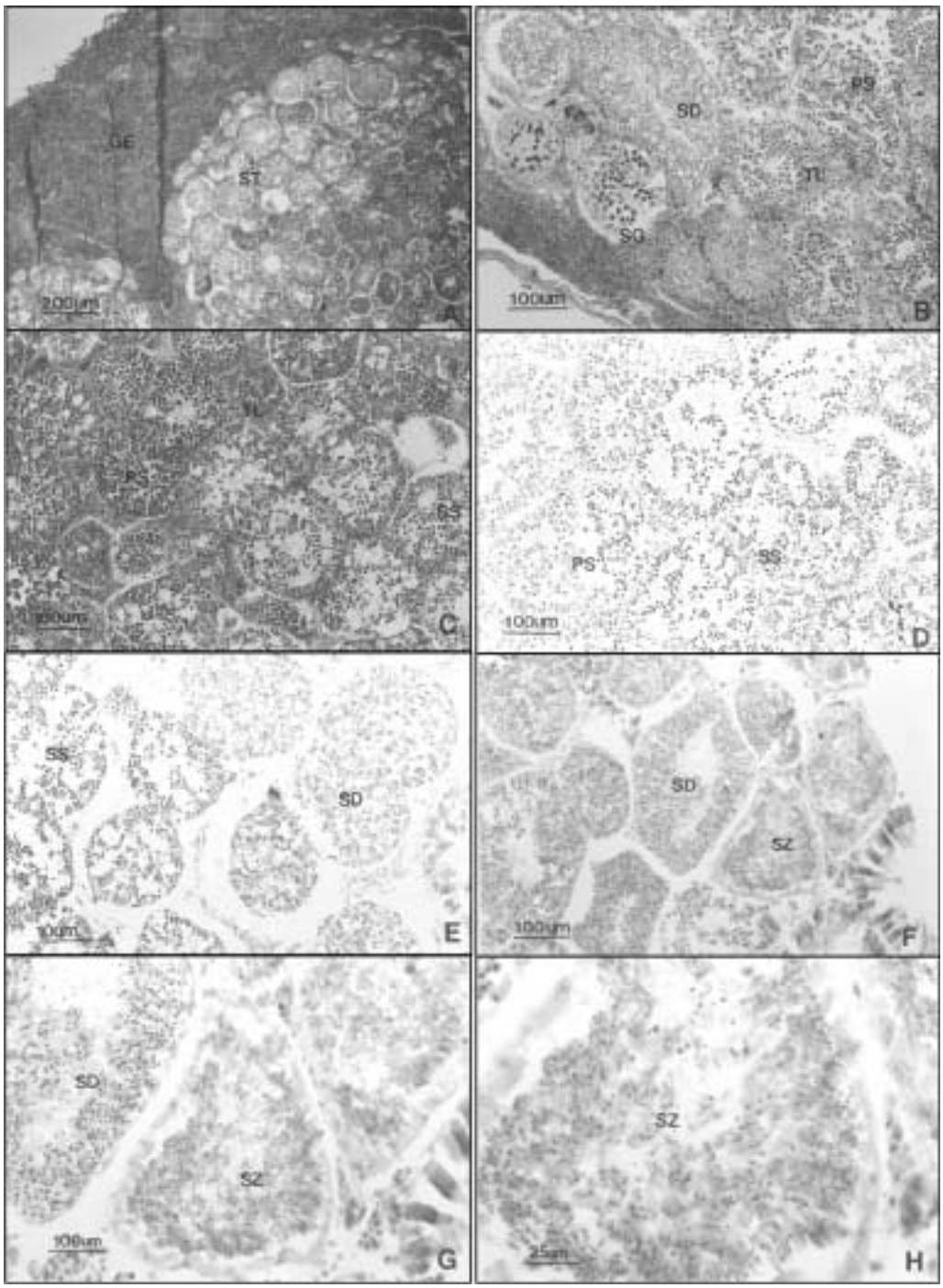

Fig. 3. Microphotographs of testis developmental phases in *Raja koreana*. (A) 63.3 cm total length (TL), 8th, Nov. 2002. Seminiferous tubules (ST) developed from germinal epithelia (GE). (B) 67.0 cm TL, 22nd, Jan. 2003. Spermatogonia (SG), primary spermatocytes (PS) and spermatids (SD) appeared in testicular lobule (TL). (C) 63.3 cm TL, 8th, Nov. 2002. PS and secondary spermatocytes (SS) appeared in Tl. (D) 81 cm TL, 5th, June, 2003. PS and SS stained by haematoxylin. (E) 77.3 cm TL, 28th, Nov. 2002. SS and SD distinct. (F, G) 35 cm TL, 45.4 cm TL, 26th, Aug. 2003. SD and spermatozoa (SZ) dense. (H) 60.0 cm TL, 26th, Aug. 2003. SZ distinct.

(Fig. 3C, D, E). The spermatoblast was full of spermatids and spermatozoa (Sz) in specimens of 35 cm TL (August, 2003) (Fig. 3F) and 77.3 cm TL (August, 2003) (Fig. 3G). Mature spermatozoa

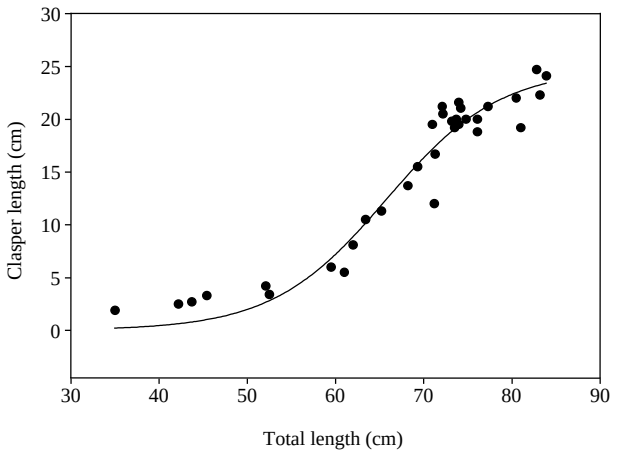

Fig. 4. Relationship between total length and clasper length of *Raja koreana*.

were ca. 10 μm (head ca. 1.7 μm) in a specimen of 77.3 cm TL (August, 2003) (Fig. 3H). Testis development always showed the above pattern, regardless of time, but maybe relating to the condition of individuals.

Clasper development was slow between 35 cm and 52.5 cm TL, increased abruptly between 59.5 cm and 71 cm TL, and thereafter decreased, showing an overall sigmoidal growth pattern (Fig. 4) [growth equation in TL: $CL = 24.8 \cdot [1+\exp\{-(TL-65.8) \cdot 6.44^{-1}\}]$ ($n = 33$, $r^2 = 0.94$, $P < 0.001$)].

Female reproductive organ

Female reproductive organs comprised ovaries, oviducts and shell gland. The ovary developed from the anterior end of the ovarian membrane, forming circular or elliptical oogonia of ca. 58～125 μm, without an ovarian lamella in a specimen of 30 cm TL (August, 2003) (Fig. 5A). Follicular cells and yolk materials were observed in oocytes in specimens of 66.5 cm TL (June, 2003) (Fig. 5B) and 65.8 cm TL (November, 2002) (Fig. 5C). Oocytes between ca. 400 to 800 μm showed only the yolk, without nucleolus or nucleus (Fig. 5B, C). Nucleus (eosin stain) of oocyte was apparent in a specimen of 68.5 cm TL (February, 2003) (Fig. 5D), and it moved to the animal pole in a ripe egg (Fig. 5E) with the yolk vesicle near the nucleolus or inner ovarian membrane in a specimen of 61.0 cm TL (November, 2002) (Fig. 5F). Thereafter, ripe eggs were ovulated to the oviduct and an egg capsule formed.

Two egg capsules were found in the oviducts of specimens larger than 70 cm TL in May and November 2003 (Fig. 6).

Discussion

In both skates and sharks, the relative size and structure of the clasper in males can be used for estimating sexual maturity and species boundaries (Pratt, 1979; Ishihara, 1987). In male of Korean skate, the claspers lengthened abruptly from 60 cm TL, peaking at 82.8 cm TL, and the maximum clasper length was 24.7 cm. It is likely that male individuals less than 60 cm TL cannot copulate owing to their small and flexible claspers. However, sexual maturity in male individuals less than 60 cm TL was detected by histological investigations. Surprisingly, a specimen of 35 cm TL had mature testis, filled with spermatids and metamorphosing spermatozoons, despite small claspers of only 1.9 cm length. Other studies showed that *Etmopterus unicolor* (Yano and Tanaka, 1989) and *Leucoraja ocellata* (Sulikowski *et al*., 2005) began to mature when the claspers started to elongate abruptly or slightly after that, whereas *Alopias superciliosus* (Chen *et al*., 1997) and *Lamna nasus* (Jesen *et al*., 2002) were mature from the point at which clasper reached their maximum length. Although some small difference was apparent in the relationship between clasper length and sexual maturity in skates and sharks, the latter in males must be related to clasper condition. Korean skate, however, showed a histologically mature testis before the clasper started to lengthen. These interesting findings may be related to male-biased relative size in specimens less than 50.4 cm TL (Kim, unpubl.), contributing to female preferences for large males (Andersson, 1994)

The histological observations of Korean skate showed the testis development to be consistent with that in bony fishes (Yasutake and Wales, 1983). On the other hand, development of the ovaries differed greatly from the latter as follows; 1)oogonia developed directly from parenchymal tissues, 2)oocytes showed various sexual developmental stages simultaneously, i.e., oocytes without a nucleus, with a nucleus, and with the nucleus moved to the animal pole (observed in one specimen of Korean skate) as in *R. pulchra* (Yeon *et al*., 1997). Reproductive cycle of elasmobranches was basically divided into three types (Wourms, 1977) as follows; 1) reproduction throughout the year, 2) a partially defined annual

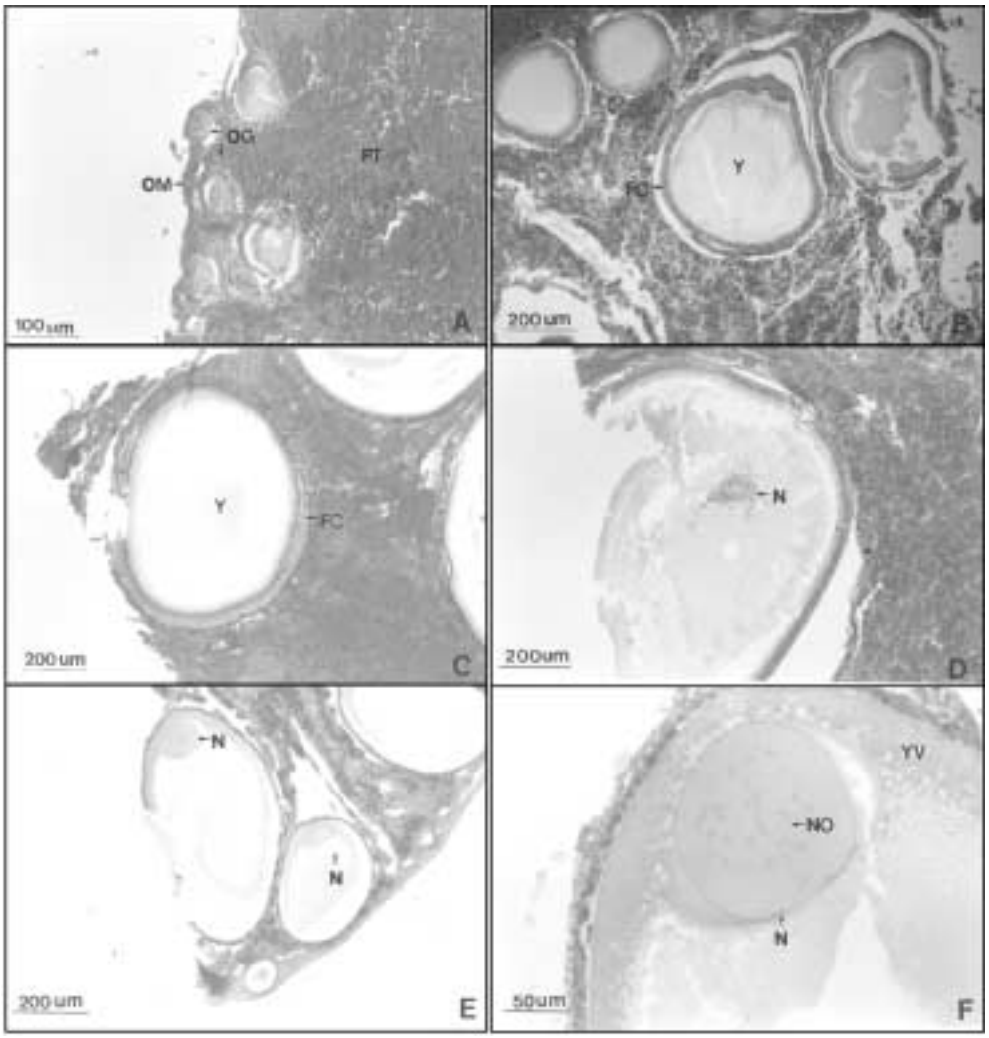

Fig. 5. Microphotographs of ovary developmental phases in *Raja koreana*. (A) 30 cm TL, 26th, Aug. 2003. Oogonia (OG) appeared at anterior end of ovarian membrane (OM). (B, C) 66.5 cm TL, 9th, June 2003, 65.8 cm TL, 12nd, Nov. 2002. Follicular cells (FC) and yolk (Y) appeared in oocytes (OC). (D) 68.5 cm TL, 19th, Feb. 2003, Nucleus (N) appeared in Y. (E) 61 cm TL, 28th, Nov. 2002. N moved to animal pole. (F) 61 cm TL, 28th, Nov. 2003. Nucleolus (NO) and yolk vesicle (YV) appeared.

cycle with one or two peaks during the year, 3) a well defined annual or biennial cycle. Among them, the males of Korean skate belong to the first type being mature throughout the year, whereas female do not conform to any of the three types because they can spawn throughout most of the year except summer. Not with standing, the females of Korean skate are most likely to belong to the second type on the basis of the time of appearance of egg capsules. This phenomenon has also been found in *R. pulchra* (Yeon *et al.*, 1997). Clearly both sexes must be remain mature for as long as possible to compensate for their low fecundity. However, several sharks, such as *Centroscymnus owstoni* and *C. coelolepis* (Yano and Tanaka, 1988), *Chlamydoselachus anguineus* (Tanaka *et al.*, 1990), and *Galeus sauteri* (Chen *et al.*, 1996) belong to the first type, regardless of sex. This is apparently associated with their habitat, all of latter living in stable deep sea. However, Korean skate lives only in the south-western sea of Korea (Jeong and Nakabo, 1997; Jeong, 1999), an unstable water body influenced by various factors, i.e., Yellow Sea Bottom Cold Water, Tsushima Current Water and South Coastal Water of Korea (Kim *et al.*, 2002). Sea temperature in this area fluctuates yearly, which makes a difference in species composition as well

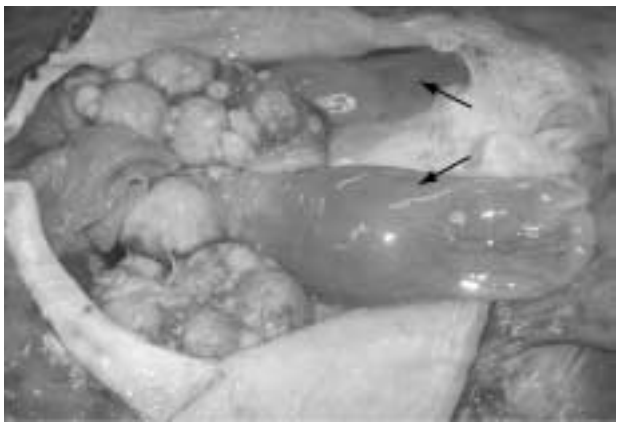

Fig. 6. Two egg capsules (arrows) of *Raja koreana* females.

as catch of anchovy (Kim *et al*., 2002).

Although information on maturity we obtained in this study would be useful for the stock management of Korean skate, additional studies on the age and growth of the species related to environmental variation are necessary for understanding their whole life cycle.

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한국산 고려홍어의 성성숙

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2002년 9월부터 2003년 12월 동안 한국 남서부해역에서 어획된 고려홍어(*Raja koreana*) 89마리를 대상으로 그들의 생식소 발달과정을 조직학적으로 조사하였다. 수컷의 교미기는 전장 35 cm에서 52.5 cm 사이에서 느리게 성장하다가 59.5 cm부터 71 cm 사이에서 갑자기 빨라졌고 이후 다시 감소하는 시그모이드 성장패턴을 보여 주었다. 다른 홍어류 및 상어류와 달리 고려홍어 수컷은 교미기가 길어지기 전에 조직학적으로 이미 성숙한 상태였다. 비록 고려홍어 암컷에서 난각이 5월과 11월에만 발견되었지만 조직학적으로 암컷은 여름을 제외한 연중 방란이 가능하고 수컷은 연중 방정이 가능한 것으로 나타났다.