ORIGINAL ARTICLE

Seasonal changes of spermatogenesis in the male sand goby *Oxyeleotris marmoratus* Bleeker, 1852 (Teleostei, Gobiidae)

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Abstract

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The annual reproductive cycle of the total 84 mature male sand gobies *Oxyeleotris marmoratus*, was investigated during March 2002 to March 2003. The specimens were obtained from the natural freshwater marsh in Pattani Province, southern Thailand. The seasonal changes in the testes were determined based on the histological characteristics during testicular development. The cranial, medial and caudal regions of testis are synchronously arranged with various stages of germ cells such as spermatogonia, spermatocytes, spermatids and spermatozoa. The germ cells are found from the periphery to the center of each seminiferous tubule. The testicular cycle of adult male *O. marmoratus* can be divided into five stages: resting, developing, mature, spawning, and spent stages. In the present study, *O. marmoratus* shows a seasonal cycle of spermatogenesis with a defined spermiation period. The highest spawning peak occurred in November 2002, and the second highest peak in May with respectively 100% and 66% of male spawning. Spawning did not take place during January to March. However, sperm production occurred throughout the year and presented three peaks of mature stage in April, June, and September. The present work describes the ultrastructure of spermatogenesis with an emphasis on the spermiogenesis. The mature sperm consists of a head without an acrosome, a short midpiece and a long flagellar tail with lateral fins. The flagellum contains an axoneme of classical form

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with 9 peripheral double microtubules. The nucleus is symmetrical with a deep basal invagination and the centriolar complex is located outside the nuclear fossa. Sperm morphology and spermatogenesis between sand goby and those of the related families are compared.

Key words : reproductive cycle, sand goby, spermatogenesis, *Oxyeleotris marmoratus*

บทคัดย่อ

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การเปลี่ยนแปลงตามฤดูกาลของกระบวนการสร้างอสุจิในปลาบู่ทราย *Oxyeleotris marmoratus*

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ศึกษาวงจรสืบพันธุ์ของปลาบู่ทรายเพศผู้ *Oxyeleotris marmoratus* ตัวเต็มวัยจำนวน 84 ตัว จากแหล่งน้ำธรรมชาติ เขตจังหวัดปัตตานี เป็นเวลาติดต่อกัน 13 เดือน ตั้งแต่เดือน มีนาคม 2545 ถึง มีนาคม 2546 จากการวิเคราะห์การเปลี่ยนแปลงของเนื้อเยื่ออัณฑะทุกเดือน พบว่าการสร้างเซลสืบพันธุ์มีพัฒนาการพร้อมกันเป็นกลุ่มตลอดความยาวของอัณฑะ การศึกษาพัฒนาการของเซลสืบพันธุ์ระยะต่าง ๆ ตั้งแต่ สเปอร์มาโตโกเนีย (spermatogonia) สเปอร์มาโตไซต์ (spermatocyte) สเปอร์มาติด (spermatid) และอสุจิ (sperm) หรือสเปอร์มาโตซัว (spermatozoa) ภายในหลอดสร้างอสุจิ (seminiferous tubule) สามารถแบ่งวงจรสืบพันธุ์ของปลาบู่ทรายเพศผู้ ออกเป็น 5 ระยะ คือ 1. ระยะพัก (resting stage) 2. ระยะพัฒนาการ (developing stage) 3. ระยะอสุจิเจริญเต็มที่ (mature) 4. ระยะปล่อยอสุจิหรือน้ำเชื้อ (spawning stage) และ 5. ระยะหลังปล่อยน้ำเชื้อ (spent stage) จากการศึกษาพบว่าปลาบู่ทราย *O. marmoratus* สร้างอสุจิเป็นฤดูกาลและมีระยะปล่อยน้ำเชื้อที่ชัดเจน โดยปลาบู่ทรายทุกตัวมีอัณฑะอยู่ในระยะปล่อยน้ำเชื้อ คิดเป็น 100 % และ 66 % ในเดือนพฤศจิกายน และเดือนพฤษภาคมตามลำดับ สำหรับระหว่างเดือนมกราคมถึงเดือนมีนาคม ไม่พบระยะปล่อยน้ำเชื้อ อย่างไรก็ตามปลาบู่ทรายชนิดนี้สร้างอสุจิตลอดปี โดยมีระยะอสุจิเจริญเต็มที่ที่ 50 % ในเดือนเมษายน มิถุนายน และกันยายน

จากการศึกษาโครงสร้างโดยละเอียดของกระบวนการสร้างอสุจิ ด้วยกล้องจุลทรรศน์อิเล็กตรอนชนิดส่องผ่าน (transmission electron microscope) พบว่าอสุจิของปลาบู่ทรายประกอบด้วย 3 ส่วน คือส่วนหัว ซึ่งไม่มีอโครโซม (acrosome) ส่วนกลาง (midpiece) มีขนาดสั้น และส่วนหางมีขนาดยาว หางมีครีบข้าง (lateral fins) แกนกลางของหางประกอบด้วย axoneme ซึ่งมีโครงสร้างหลักคือ 9+2 ไมโครทิวบูล (microtubule) สำหรับนิวเคลียสของอสุจิมีลักษณะสมมาตร ส่วนท้ายของนิวเคลียสเป็นร่องลึกเรียก nuclear fossa อสุจิของปลาชนิดนี้มี centriolar complex อยู่นอก nuclear fossa ซึ่งแตกต่างจากปลาบู่ทรายบางชนิด การศึกษาครั้งนี้ได้เปรียบเทียบลักษณะของอสุจิและกระบวนการสร้างอสุจิของปลาบู่ทรายปลาชนิดต่าง ๆ ที่อยู่ในตระกูลใกล้เคียงกัน

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Gobioids comprise a large group of marine, freshwater or estuarine fishes. Among them, sand goby *Oxyeleotris marmoratus*, is one of the most important freshwater gobiid for commercial aquaculture. Because of the tender flesh and good flavour of *O. marmoratus*, the market demand for this species is increasing. Today the fish farming of sand goby *O. marmoratus* in Thailand is being expanded but the production is limited. Although the reproductive cycle of both male and female fishes observed from histological changes of gonad have been studied by many authors (Htun-

Han,1978; Loir *et al*., 2001; Rosenblum *et al*.,1987; Rosenblum *et al*., 1994), prior studies on reproductive cycles of goby are rare (Kaneko and hanyu, 1985) and data on sand goby in Thailand are not available. Among Gobioids, the histology of the reproductive cycle of sleeper *Eleotris acanthopoma*, an estuarine gobioid fish, has been investigated (Wang *et al*., 2001). In addition, the annual changes of seminal vesicle of gobiid fish *Zosterisessor ophiocephalus* has been studied at the ultrastructural level (Lahnsteiner and Patzner, 1990).

To understand the reproductive cycles, studies on the timing of spawning, gonad development, sex ratio, fecundity and even ultrastructure of spermatogenic cells must be included. The study on the spawning in male is important particularly in fish species in which the male undertakes parental care (Maconato *et al*., 1996). In male *O. marmoratus*, the spawning occurs in a nest and the male cares for the fertilized eggs (Amornsakun *et al*., 2002), similar to Blennidae and Opistognathidae (Breder and Rosen, 1996). Male teleost produce sperm either continuously or discontinuously depending on the species. Moreover, variable intervals may occur between the completion of spermatogenesis and the initiation of spawning (Todd, 1976; Borg, 1982), or spawning may proceed directly upon the completion of spermiogenesis (Ruby and MacMillan, 1970). Thus, it is necessary to investigate the reproductive biology of the male in various respects including the seasonal cycles of testicular activity and spermatogenesis.

In teleosts, spermatozoa and spermatogenesis have been studied at the ultrastructural level in a number of different families, for instance in Mugilidae (Brusle, 1981), Salmonidae (Billard, 1983), Cyprinidae (Baccetti *et al*.,1984), Blennidae (Lahnsteiner and Patzner, 1990), Gadidae (Lahnsteiner *et al*., 1994), Pimelodidae (Santos *et al*., 2001), and Scombridae (Abascal *et al*., 2002). Within the family Gobiidae the sperm ultrastructure has been described only in a few gobiid species such as *Periophthamus papilio*, the mudskipper (Mattei, 1970). On the other hand, numerous investigators have studied the morphology and cytology of seminal vesicles or sperm duct gland to find some macro and microevolutionary similarities between species groups of gobies (Cinquetti, 1997; Fishelson, 1991). The teleost spermatozoa exhibit a broad range of morphological structure among species and can be used for classification. In cyprinidae, different species belonging to the same genus show significant differences of sperm ultrastructure (Baccetti *et al*., 1984). In tunas *Thunnus thynnus* and *Euthynnus alletteratus* the ultrastructural characteristics of their spermatozoa show small species-specific divergences that could be useful in systematics (Abascal *et al*., 2002). Although the sand goby has a high commercial value for fisheries, there is no information on the fine structures of spermatogenesis. Therefore, it is necessary to study the spermatogenic cells of this species at the ultrastructural level.

This study investigates the seasonal histological changes in sand goby testis collected from the natural freshwater marsh in Pattani Province. Histological analysis of the testicular stages, cycle and the timing of spawning are also investigated. In addition, the ultrastructural characteristics of spermatogenic cells during spermatogenesis are described to increase the current knowledge of gobiid spermatogenesis and sperm morphology.

Materials and Methods

A total of 84 adult male gobies *O.marmoratus* with the total length ranging from 20-28 cm were collected monthly during March 2002 to March 2003. The live specimens were obtained from the natural freshwater marshes in Pattani Province and taken to the Laboratory at the Department of Biology, Prince of Songkla University, Hat-Yai. After anesthetization with ice, the fish were weighed and the total length measured. The testes were quickly removed and fixed in the fixative for preservation.

Tissue preparation for light microscopy (LM)

Fragments of the cranial, medial and caudal regions of testis were fixed in the Bouin's fluid for 8-12 hrs, dehydrated in graded ethanol and embedded in paraffin. Serial sections were stained with Harris's Hematoxylin and Eosin. Histological char-

acteristics during gonadal development were examined from the sections of cranial, medial and caudal regions of six testes from each monthly sample. To determine the annual seasonal changes, the testicular development was defined according to the relative abundance of the six stages of spermatogenic cells: primary and secondary spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa. The reproductive cycle of male sand goby is divided into five stages: resting, developing, mature, spawning, and spent (modified from Loir *et al.,* 2001 and Billard, 1983).

Tissue preparation for transmission electron microscopy (TEM)

For the fine structural studies, the testes were cut into small pieces and fixed for 24 hr at 4°C in 0.1M phosphate buffer containing 4% paraformaldehyde pH 7.3 and washed in phosphate buffer. After postfixation in 1% osmium tetroxide, the specimens were dehydrated in an increasing concentration of ethanol and embedded in Epon 812. Semithin and ultrathin sections were cut with an ultramicrotome model MTXL. The ultrathin sections were stained with uranyl acetate and lead citrate. The specimens were then examined under a JEOL 100 transmission electron microscope at the Electron Microscope Unit, Department of Pathology, Faculty of Medicine, Prince of Songkla University.

Results

Seasonal changes in testes

The testes of *O. marmoratus* are paired and elongated structures located ventral to the swim bladder. The testis tissue is composed mainly of seminiferous tubules. The wall of the seminiferous tubules is characterized by the presence of germinal cysts, each of which contains spermatogenic cells. In the mature testis, various stages of germ cells such as spermatogonia, spermatocytes, spermatids and spermatozoa can be found from the periphery to the center of each seminiferous tubule. In *O. marmoratus*, the spermatogenic cells in each germinal cyst are in the same stage of development and the different cysts are found along the lumen (Figure 1b and 1c). The testicular development observed from the cranial, medial and caudal regions of testis is synchronously arranged. The seasonal changes in the testis were defined based on the criteria of the histological characteristics and the relative abundance of spermatogenic stage as indicated in Table 1. The testicular cycle could be divided into resting, developing, mature, spawning and spent stages.

The histological characteristics of each stage in the testis are as follows.

Resting stage: During this stage, the testes are morphologically small and show no spermatogenic activity. The resting testicular tubules exhibit a large number of primary and secondary spermatogonia congregating along the inside wall of the seminiferous tubules. A few primary spermatocytes are observed. The lumen is narrow and usuall clear. In some tubules, unshed sperms are present in the lumen (Figure 1a). Resting stage is infrequently observed in December and gradually increases until March, and then becomes the developing stage (Figure 2).

Developing stage: Testis in this stage gradually increases in size and the walls of seminiferous tubule are filled with germinal cysts of all stages (Figure 1b). Primary and secondary spermatogonial cysts are apparently reduced, while those of primary and secondary spermatocytes increase and later decrease as they mature into spermatids. The number of spermatids and spermatozoa greatly increase in the late of developing stage. In this study, the developing stage occurs throughout the year, except in June, August, October and November (Figure 2).

Mature stage: Testis becomes fully mature and physiologically ready to spawn. The maximal quantity of spermatozoa is observed in the lumen of the seminiferous tubule so that the entire lumen is filled with spermatozoa (Figure 1c). Few spermatogonia and spermatocytes are present in the wall of the seminiferous tubule. The maturity cycle of *O. marmoratus* fluctuates. There are three similar

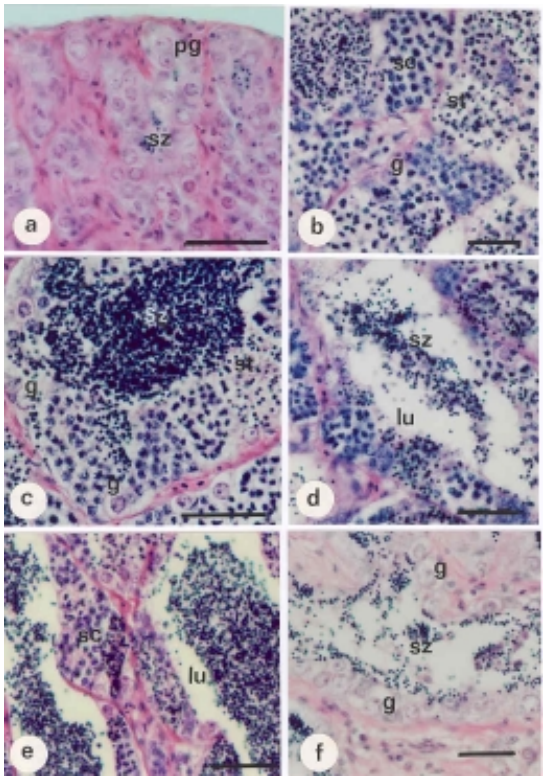

Figure 1. Transverse sections of *O. marmoratus* testes show the various stages of testicular development. a. Resting stage, b. Developing stage, c. Mature stage, d-e. Spawning stage, and f. Spent stage. (H&E).
g - spermatogonia; lu – lumen; pg - primary spermatogonia; sc - spermatocytes; st - spermatids; sz - spermatozoa. Scale bar = 150 µm.

Table 1. Staging criteria employed for testicular development in *O. marmoratus.*

Stage	Relative abundance of various spermatogenic cells in testis					
	1°spermatogonia	2°spermatogonia	1°spermatocytes	2°spermatocytes	spermatids	spermatozoa
Resting	+ + + +	+ + +	+	-	-	-
Developing	+ +	+ + +	+ + +	+ +	+ + +	+ + +
Mature	+	+	+ +	+ +	+ + +	+ + + +
Spawning	+ +	+ +	+	-	+ +	+ + +
Spent	+ + +	+ +	-	-	+	+ +

+ to + + + + indicates the degree of abundance; - , means not found.

peaks, in April, June, and September (Figure 2).

Spawning stage: A number of spermatozoa have been shed leaving the lumen with some empty spaces (Figure 1d and 1e). The primary and secondary spermatogonia begin to increase in number. Primary spermatocytes are found in some germinal cysts, but secondary spermatocytes are not present at this stage. In November, all sand goby

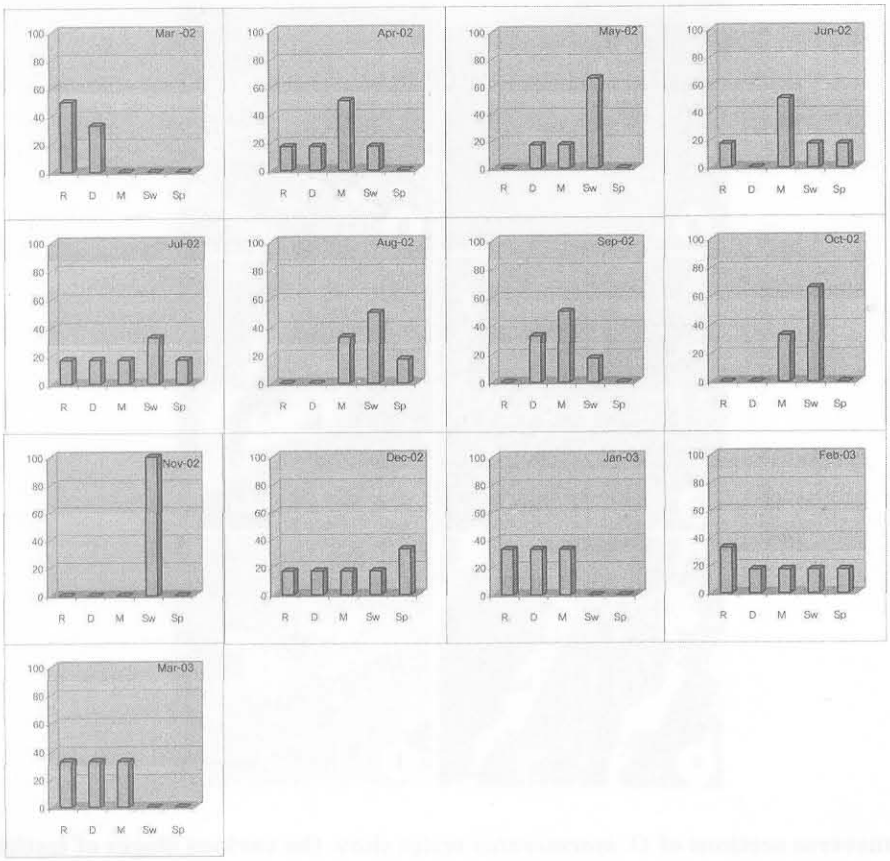

Figure 2. Testicular development of the sand goby *Oxyeleotris marmoratus* during March 2002 to March 2003, base on a total number of 6 samples/month
R = resting stage; D = developing stage; M = mature stage; Sw = spawning stage; Sp = spent stage

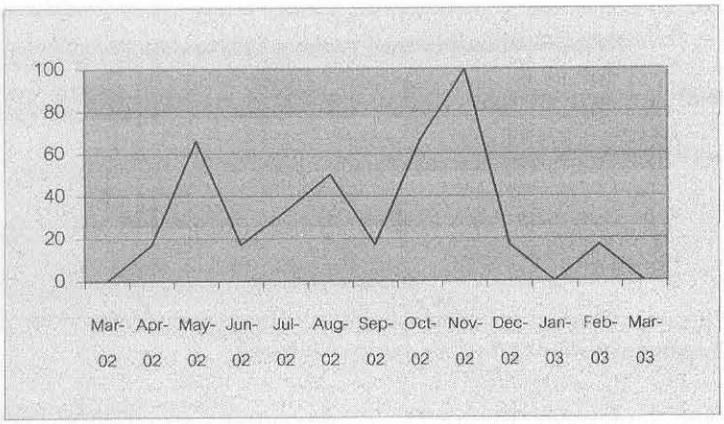

Figure 3. Seasonal changes in spawning stage of *Oxyeleotris marmoratus* testes during March 2002 to March 2003.

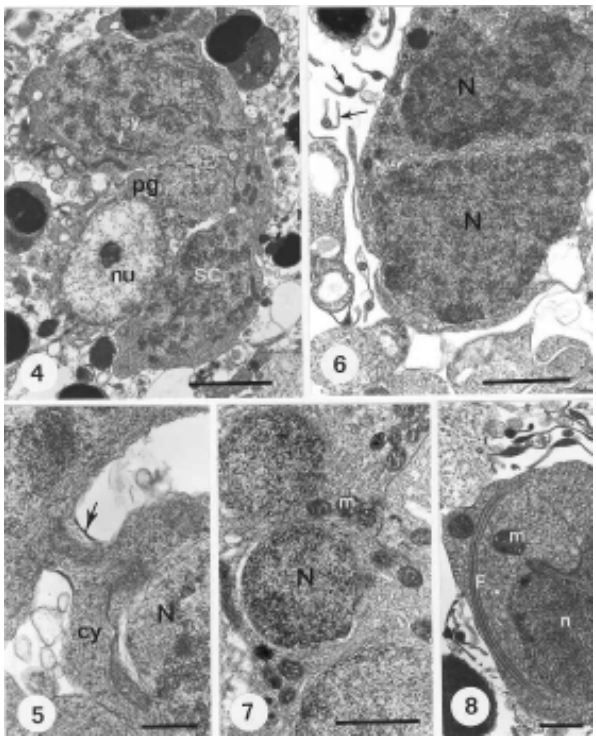

Figure 4. Transmission electron micrograph of primary spermatogonia (pg) and primary spermatocyte (sc). The nucleus of the primary spermatogonia contains mostly euchromatin and a nucleolus (nu). The primary spermatocyte shows denser nucleus with synaptonemial complex (sy). Scale bar = 4 µm.

Figure 5. Two spermatocytes connected by cytoplasmic bridge (arrow). cy - cytoplasm; N = nucleus. Scale bar = 5 µm.

Figure 6. Spermatocyte with two nuclei (N). Note the cross section of sperm tails with lateral fins (arrow). Scale bar = 2 µm.

Figure 7. Early spermatids with round nucleus (N) and numerous mitochondria (m) in the cytoplasm. Scale bar = 2 µm.

Figure 8. Early spermatid showing the flagellar formation in the cytoplasm. F - flagellum; m - mitochondria; n - nucleus. Scale bar = 1 µm.

testes are in the spawning stage and there are no other stages found in this month (Figure 2).

Spent stage: The spermatids and spermatozoa are less abundant, and there are many empty spaces in the lumen (Figure 1f). Spermatogonia further increase in number indicating the onset of new development. Spermatocytes are absent or few in this stage. The spent period of *O. marmoratus* testes apparently occurred in December after the spawning time in November (Figure 2).

The testicular development in *O. marmoratus* testes of each month during March 2002 to March 2003 is presented in Figure 2, and the spawning period of *O. marmoratus* are shown in Figure 3. The results show that the spermatogenic activity was low during January to March, and the devel-

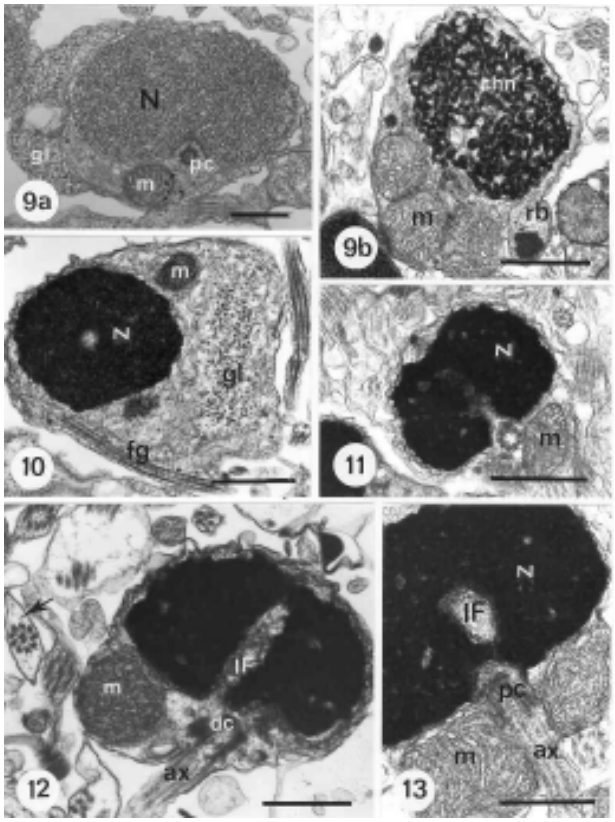

Figure 9. ***O. marmoratus*** **spermatids showing the nuclear condensation. 9a : nucleus at granular stage, 9b : nucleus at lamellar stage. chn – nuclear chromatin; gl - glycogen; m - mitochondria; pc - proximal centriole; rb - residual bodies. Scale bar = 1 µm.**

Figure 10. Spermatid with electron dense nucleus (N) and flagellar formation. Note the abundance of glycogen granules (gl) in the cytoplasm. fg - flagellum; m - mitochondria. Scale bar = 1 µm.

Figure 11. Longitudinal section of the head region of late spermatid showing the electron dense kidney shaped nucleus (N). m - mitochondria. Scale bar = 1 µm.

Figure 12. Anacrosomal spermatozoon of ***O. marmoratus*** **showing the nuclear fossa (IF) and the forming of large mitochondria (m). Note the cross section of flagellum with the lateral fin (arrow). ax – axoneme; dc - distal centriole. Scale bar = 0.6 µm.**

Figure 13. Frontal section of the head region of a mature spermatozoon showing a symmetrical nucleus. The centriolar complex (pc) located outside the nuclear fossa (IF). ax - axoneme; N - nucleus. Scale bar = 0.5 µm.

opment of spermatogenesis was greatly increased in April to May. The highest percentage of mature stage (50%) occurred in April, June and September. Sperm production in *O. marmoratus* with the highest peak (100%) took place in November, and the second peak (66%) occurred in May. The results also show that the spawning stage did not take place in January and March.

Ultrastructure of spermatogenic cells

The ultrastructural characteristics of various stages of spermatogenic cells in *O. marmoratus* are described as follows:

Spermatogonia: Spermatogonia possess a large round nucleus located in the center. The nucleus shows lucent dispersed chromatin with one or two distinct round nucleoli. The cytoplasm contains few organelles and a few scattered mitochondria (Figure 4).

Spermatocytes: Primary spermatocytes originated from the last generation of secondary spermatogonia. The distinctive changes from spermatogonium to spermatocyte are the decrease in size and increase in nucleus to cytoplasm ratio. The nucleus is variable as the meiosis proceeds. Primary spermatocytes can be easily characterized by the synaptonemial complex in the nucleus (Figure 4). It has a dense cytoplasm containing small rough endoplasmic reticulum and Golgi complex. Cytoplasmic bridge is often seen at this stage (Figure 5). Secondary spermatocytes are produced by the first meiotic division of primary spermatocytes. They are spherical in shape with the concentric nucleus (Figure 6). The nucleus is round and contains a number of large clumps of heterochromatin blocks. The cytoplasm is granulated containing moderate sized mitochondria.

Spermatids: Spermatids are smaller than secondary spermatocytes. During spermiogenesis, these cells undergo cellular changes. This process involves in a reduction of both nuclear and cytoplasmic volume. At the ultrastructural level, nuclear condensation and the formation of the middle piece are observed. At the early stage, young spermatids contain a round centrally located nucleus. The nuclear chromatin is homogeneous and displays a finely granular appearance. Cytoplasmic and organelles are accumulated at the cellular pole opposite to the nucleus. Mitochondria increase in size and congregate at one pole of the cell (Figure 7). The axoneme is seen in the cytoplasm and wraps around the nucleus as its length increases (Figure 8).

In the middle stage of the spermatids, the nucleus decreases in size and becomes more compact with increasing amount of thick chromatin filaments (Figure 9a and 9b). The cytoplasm shows a few smooth and rough endoplasmic reticulum, numerous ribosomes, and glycogen granules (Figure 10). The excess portions of cytoplasm are cast off as residual bodies into the lumen (Figure 9b). Consequently, the previously scattered mitochondria aggregate to the posterior of nucleus, and eventually group together around the axonemal base (Figure 11). The axoneme arises from the distal centriole.

Spermatozoa: The mature spermatozoon of *O. marmoratus* consists of a head without an acrosome, a midpiece and a flagellum. The head contains an electron-dense nucleus. The nucleus has a symmetrical kidney shape of about 1.5 μm in length and 2.5 μm in width with a basal invagination (Figure 12). Just behind the nucleus is the middle piece that is short and contains a few mitochondria surrounding the centriole. The centriolar complex is located outside the nuclear fossa (Figure 13). The flagellum is long and contains microtubules in 9+2 axonemal arrangement. The plasma membrane of flagellum is extended as a fin (Figure 12).

Discussion

Seasonal changes

The stages of reproductive cycle of male sand goby *O. marmoratus* is modified from Loir *et al.* (2001) and Billard (1983) and divided into five stages: resting, developing, mature, spawning, and spent. During the resting period, lasting from January to March, most testes possess germinal cysts of spematogonia and spermatocytes, which indicate the low spermatogenic activity in the testis. For the mature stage, it is obviously seen during April to September. This reveals that spermiation occurs several times a year. The beginning of the spawning stage is marked by an increased activity in conversion of spermatid to spermatozoon. During the spawning period in sand goby, a number of spermatogonia are found in the lumen and the testicular wall increases in thickness as the spawning period progresses. The small nests of dormant spermatogonia found in the testis after spawning

period are evidence of the recycle of reproduction. The spawning period of *O. marmoratus* begins in October and exhibits the high peak in November, which is the rainy season in southern Thailand. Meanwhile, the tide during October to November is highest and water temperature is lower than in other months. In an estuarine gobioid *E. acanthopoma*, the spawning period is stimulated by increasing water temperature (Lahnsteiner and Patzner, 1990), while this study shows that spawning of *O. marmoratus* takes place during the low water temperature. The different reproductive strategies between two species are related to the distinction between the environments of freshwater and brackish water. However, in the summer spawner, *Sardinops melanostictus,* water temperature may play an important role in both initiating and terminating the spawning season (Patio, 1997), whereas in an autumn spawner, *Acheliognathus rhombea*, both the lower temperature and shorter daylight had great effects in increasing the gonadosomatic index (GSI) and plasma steroid level (Matsuyama *et al*., 1990). Physiologically, the reproductive processes in fish are controlled by environmental factors, mainly photoperiod, water temperature and endogenous hormone (Shimizu *et al*., 1994). It is suggested that, even a slight local variation in environmental conditions can have a determining role in dictating sexual activity for fish species (Russo *et al*., 2000). From this study, the spawning period of male sand goby occurred during high tide period and it is strongly correlated to the spawning period in female sand goby collected from the same area (authors, in press).

The findings of the present study on sexuality and reproduction of the sand goby provides an important basis for further investigation on the reproductive biology of this species and on its propagation in captivity.

Ultrastructure of spermatogenic cells

Although the ultrastructural characteristic of the spermatozoa is not as useful in solving phylogenetic relationships as in other animal groups (Jamieson, 1991), fishes possess a specific sperm morphology that is useful in characterizing general uniform pattern to the whole family (Baccetti *et al*., 1984) and morphological differences between species can also be used in classification. Among the teleostei, there are two main morphological sperm types distinguished, the type I spermatozoon differs from type II in that the centriolar complex lies outside of the nuclear fossa or eccentric to the nucleus and the flagellar axis is parallel to the nuclear base (Mattei, 1970). In this study *O. marmoratus* sperm can be classified as type I since the centriolar complex is outside the nuclear fossa. Spermatogenesis of fish, like that of all vertebrates, consists of three general processes: meiosis, spermiogenesis and spermiation. Basicly, the primary spermatogonia undergo a series of mitotic divisions and become secondary spermatogonia. The mitotic division of secondary spermatogonia gives rise to primary spermatocytes, followed by meiotic division for secondary spermatocytes. Thus, a single spermatogonium entering meiosis eventually yields four spermatozoa. In *O. marmoratus,* spermatogenesis is synchronized and spermiogenesis is completed inside the spermatocysts. Its spermatogenesis is fundamentally similar to that found in other vertebrates (Nagahama, 1983; Callard, 1991). The spermatogonium is characterized by its large central, vesicular nucleus and a distinct nucleolus. Axoneme formation is apparent in the cytoplasm of the early spermatid. Since few data are available on spermiogenesis in species of Gobiidae, the spermatid differentiation or spermiogenesis in this study is divided into three prominent stages modified from the criteria used in other families that were proposed by previous authors (Lahnsteiner and Patzner, 1990; Manni and Rasotto, 1997). The results show that the main ultrastructural changes of spermatid during spermiogenesis of *O. marmoratus* follow the basic pattern which occurrs in other fish species (Brusle, 1981; Sprando and Russell, 1988; Todd, 1976). However, the accumulation of glycogen granules in the cytoplasm of the middle stage spermatid of the sand goby is remarkable and easily recognizable. Chromatin condensation occurs synchronously throughout the nucleus of the spermatid, whereas the nuclear chromatin condensation in *Lepomis macrochirus* was initiated from a re-

gion near the implantation fossa leaving a clear area at the opposite pole (Sprando and Russel, 1988). The gobioid sperm have a broad range of variation even within a family. Of all spermatozoal characteristics, the arrangement and the number of the midpiece mitochondria are highly variable taxonomically. In *Opisthognathus whitehursti*, the midpiece of mature spermatozoa contained four spherical mitochondria approximately of the same size (Manni and Rasotto, 1997), and in the gobioid *Hypseleotris galii* and *Periophthalamus papillo*, the midpiece has a single mass of mitochondrial material (Mattei, 1970). By contrast, the midpiece of the sand goby sperm is composed of a few mitochondria. In cyprinid fishes, the mitochondrial number was suggested as an interesting character for phylogenetic arrangement (Baccetti *et al*., 1984).

The mature spermatozoa of *O. marmoratus* exhibit the configuration of the uniflagellate anacrosomal aquasperm typically found in externally fertilizing fishes (Jamieson, 1991). Contrary to fish in Scombridae (Abascal *et al*., 2002), fins are present on the mature flagellum of the sand goby sperm as an extension of plasma membrane. In addition, the shape of nucleus and the localization of the centriolar complex of *O. marmoratus* are completely different from those of goby *Periophthamus papillio*. The nucleus of *O. marmoratus* is symmetrically kidney shaped with a deep basal fossa while that of *Periophthamus papillio* is asymmetrical and its basal fossa contains the proximal centriole (Mattei, 1970). From this study, it is concluded that *O. marmoratus* spermatogenesis has a great deal of similarity to that of other species of related families. However, the species-specific characteristics of spermatogenic cells of the sand goby *O. marmoratus* are considered as the taxonomy significant.

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