Bull. Mar. Res. Inst.
Cheju Nat. Univ., 17 : 23~32, 1993

濟州大 海洋硏報, 17 : 23~32, 1993

Changes in the testicular structures with the testicular development of damselfish, *Chromis notatus*(Temminck et Schlegel)

Young-Don Lee · Ee-Yung Chung* · Taek-Yuil Lee**

Marine Research Institute, Cheju National University, Cheju-do 695-810, Korea
**Department of Marine Development, Kunsan National University, Kunsan 573-360, Korea*
***Department of Marine Biology, National Fisheries University of Pusan, Pusan 608-737, Korea*

자리돔, *Chromis notatus*(Temminck et Schlegel)의 精巢發達에 따른 精巢構造의 變化

李榮敦 · 鄭義泳* · 李澤烈**

濟州大學校 海洋硏究所, *群山大學校 海洋開發學科, **釜山水産大學校 海洋生物學科

Changes in the testicular structures and gonadosomatic index(GSI) of *Chromis notatus* was histologically investigated. Specimens of damselfish were monthly collected by the Jari-fishing nets at the coastal area around Seogwipo, Cheju-do, Korea from February 1990 to September 1991. Sexuality of the species was dioecieous. The testis was paired, located along the vertebrae in the dorsal posterior part of the abdominal cavity, and was bilateral asymmetrical. The testis consisting of a pair of lobular structures in the right and left was united in the posterior seminal vesicle. Cortex of the testis was composed of several testicular lobules and medulla was composed of many sperm ducts connected with the lobules. The course of maturation in the present species proceeded synchronously in all cells within a cyst, but it was different more or less from lobule to lobule. Mature spermatozoa were accumulated in the lumen of the lobules. A small number of spermatogonia remained as residual spermatogonium in the resting state at the resting stage as a reserver for the next generation of germ cells. Spermatogenesis was shown active in the early development stage, but development of interstitial cells were very poor at this stage. Maximal development of apparently active interstitial cells occured in the mature stage during the period from May to September but secretory activity had not been observed clearly in them. It is also suggested that in the early phases of testicular growth, interstitial cells probably does not particularly medidate pituitary stimulation of spermatogenesis. Activity of the intralobular cyst cells changed clearly along with their sexual maturation. They were thin, elliptical or triangular shape, their nucleus and cytoplasm increased in size during the mature and ripe stage, while they decreased markedly in size and showed strong affinity to haematoxylin after breeding. Secretory activity of these cells had not been observed clearly in them also. Environmental factors seemed to stimulate testicular growth and maturity as judged from the more advanced sexual stages of the testes. The maturation of the testis was supposed to be induced by longer day length (photoperiod) and higher water temperature, and to reach the spent period. GSI began to increase from March, starting period of longer day length and then, it gradually increased from April, and reached the maximum value in June and August when water temperatures were higher water temperature, the longest day length. Therefore, the testis development of this species is closely related to environmental factors.

Key words : 자리돔(*Chromis notatus*), 정소구조(testicular structure), 간질세포(interstitial cell), intralobular cyst cell, 환경요인(environmental factors)

INTRODUCTION

The testis is an extremely heterogeneous organ, containing nmerous compartments and cell types(Russell et al, 1990). The course of maturation in the male fish proceeds in all lobules in a testis. Within a cyst all of the germ cells is different from cyst to cyst in the testicular lobule.

In the amniotes the testecular seminiferous tubules contain Sertoli cells which extend from the basal membrane of the lobule to its lumen and exhibit a close association with the developing spermatids (Roosen-Runge, 1962).

Interstitial tissue of fish testes consist of a variety of cell types including interstitial cell, fibroblasts, and components of the blood and lymphatic systems.

It was reported that the interstitial tissue distributed in the area between the lobules, contained distinct androgen - secreting.

Leydig cells during the periods of testicular endocrine activity(Marshall and Loft, 1956; Marshall, 1960; De Wolfe and Telford, 1966; Nicholls and Graham, 1972; Gresik et al., 1973; Stanley et al, ; 1965) ascertained that the glandular interstitial cells of *Gobius paganellus* were steroid prodcing cells which are homologous with the Leydig cells in mammalian testis.

The intralobular cyst cell showed cytoplasmic changes during the period of spermiogenesis(Robertson, 1958; Hiroi and Yamamoto, 1968). It was also reported that the intralobular cyst cell of the testes in lower vertebrates, such as Pisces and Amphibia, was belived to the homolog of the Sertoli cell having a nutritive function in higher vertebrates(Stanley et al., 1965; Loft, 1972; Nicholls and Graham, 1972; Gresik et al., 1973). Up to now, however we could not find out any reports which dealt with the seasonal changes of the activity and development of these cells associated with the spermatogenesis in the testis of damselfish, while a paper had dealt with the reproductive cycle(Lee and Lee, 1987) of this species.

Therefore, the purpose of this study is to understand changes in various cells(germ cells, intralobular cyst cell, interstitial cell) in the testicular lobules by histlolgical method, and the annual changes in GSI with the environmental factors.

MATERIALS AND METHODS

Specimens of damselfish, *Chromis notatus* were collected by the Jari-fishing nets at the coastal area around Seogwipo, Chejudo, Korea(Fig. 1), for one year, from February 1990 to September 1991.

A total of 248 individuals of damselfish were used for the study. The fishes were transported alive to the laboratory where serveral measurements(total length, standard length, total weight, and testis weight) were recorded for each fish. The measurement of total length and standard length were made to the nearest 0. 1㎝ and the total weight to the nearest 0. 1g. After the testes were removed, quickly weighed on a torsion balance(accurate to the nearest 0. 0005g), and cut into small pieces.

Analysis of the gonadal phases was made by light microscopical examination of histological preparations. The tissues were subjected to standard histological procedures(dehydration in alcohol and embedded

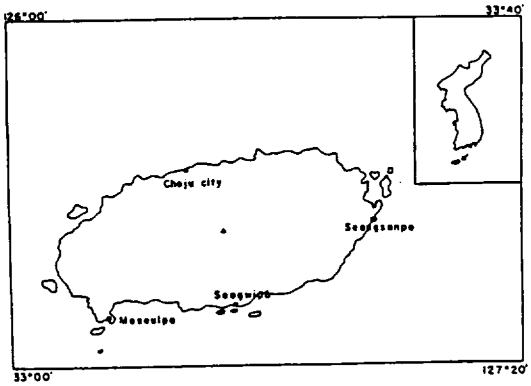

Fig. 1. Costal area around Seogwipo where the specimens were collected.

in paraffin). Embedded tissues were thin sectioned (5~8μm) on a rotary microtome. Sections were mounted on glass slides, stained with Hansen's hematoxylin – 0.5% eosin, Mallory's triple stain and PAS stain, and examined using light microscopy.

In order to make quantitatively clear the degree of the testes, gonadosomatic index (testis weight×100/body weight) was estimated for each fish.

RESULTS

External feature and structure of the testis

As shown in Fig. 2, the testis of *Chromis notatus* was paired, located along the vertebrae in the dorsal posterior part of the abdominal cavity, and was bilateral asymmetrical.

The testis consisting of a pair of lobular structures in the right and left was united in the posterior seminal vesicle. Cortex part of the testis was composed of several testicular lobules and medulla was composed of many sperm ducts connected with lobules.

Associated with the progress of maturation, the testis transformed from slender bodies into broad, thick and elongated ones, at this time, its testis was milky white in color.

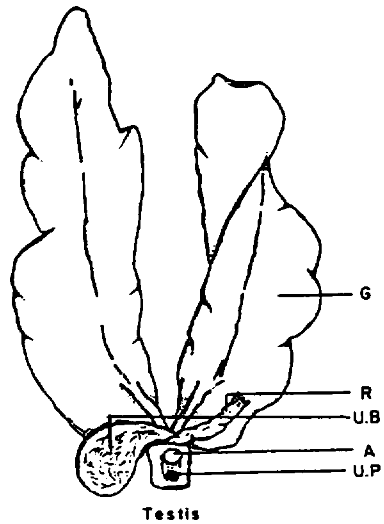

Fig. 2. Ventral view of the testis of *Chromis notatus*.
A : Anus, G : Gonad, R : Rectum,
UB : Urinary bladder
UP : Urinogenital pore

Relationship between annual changes in gonadosomatic index (GSI) and environmental factors.

The annual changes in GSI (testis weight ×100/body weight) were shown in Fig. 3.

In March when water temperatures were very low, starting the period of longer day length the mean value of GSI was 0.6, but it gradually increased from April, and rapidly increased in May, reached the maximum value (6.10) in June and August when water temperatures were higher water temperature.

Thereafter, those were slightly decreased from September to February (lower values) without any evident variation.

Therefore, the testis development of this species is closely related to environmental factors.

Changes in the germ cells, intralobular cyst cells and interstitial cells in the testes during the reproductive cycle

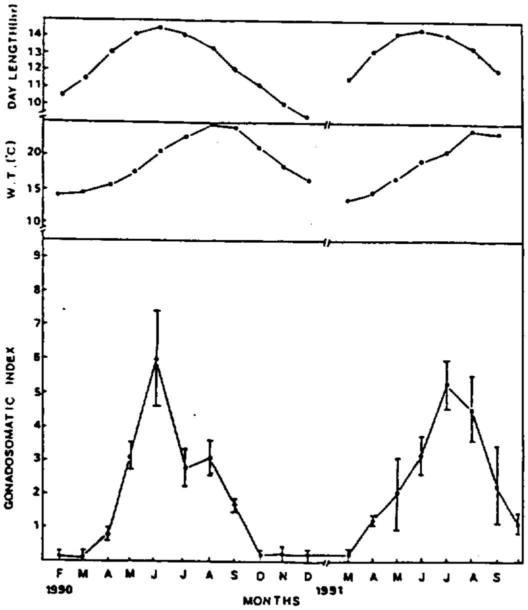

Fig. 3. Annal changes of the gonadosomatic index in male, mean mater temperature and day length from February 1990 to September 1991. Symbols and bars indicate the mean and the standard errors, respectively. W. T. represent water temperature of Seogwipo area.

The testes of immature fishes collected in March were in the early growing testis, most of the germ cells were the spermatogonia and a few spermatocytes. The spermatogonia were found to occur singly or in clusters of three to six cells, and usually surrounded by the intralobular cyst cells.

The nucleus of the singly located spermarogonium caried from round to elliptical in form, while that of the clustered cells was generally elliptical in form. At this time, the testis were composed of lobules surround by the connective tissue wall.

The lobules were filled with the intralobular cyst cells, and clusters of interstitial cells were present in interlobular spaces (connective tissue). The intralobular cyst cells are of various shapes : triangular, oval, spindle or oblong, the mean its cell size was 9.5±0.10μm. The nucleus of the cell take a dark colour with haemastoxylin, while the cytoplasm was faintly stained (Figs. 4A, B). At this stage, the interstitial cells were found to occur from three or nine cells. These islets of interstitial cells appeared to communicate with each other through narrow, interlobular connuctive tissue corridors (Fig. 4A). The mean in-terstitial cell size was 7.7±0.50μm and the mean nuclear size was 3.75±0.15μm.

The fishes collected in May had the testes in the late growing stage. The spermatogonia, large in size and singly located were still present in the lobules, and a certain number of cysts composed of the primary, secondary spermatocytes and spermatids were found in the lobules.

With increasing growth, several intralobularcyst cells were also visible, most of these cells situated along the lobule wall had each a nucleus of oval or elliptical shape. The mean its cell size was 10.0±0.05μm (Figs. 4C, D).

Interstitial cells were much better developed than those in previous stage. These cells were compactly arranged forming large aggregation, the mean interstitial cell size was 8.20±0.2μm and the mean nuclear size was 3.80±0.10μm in this stage. Their cell boundaries were not visible in the aggregation (Fig. 4C).

At this stage, a few small capillaries around connective tissues were found among the interstitial cells in the interlobular spaces.

In the testes of mature fishes collected during the period from May to August, spermatogonia and primary and secondary spermatocytes decreased in number, while spermatids and spermatozoa increased in number in the lobules.

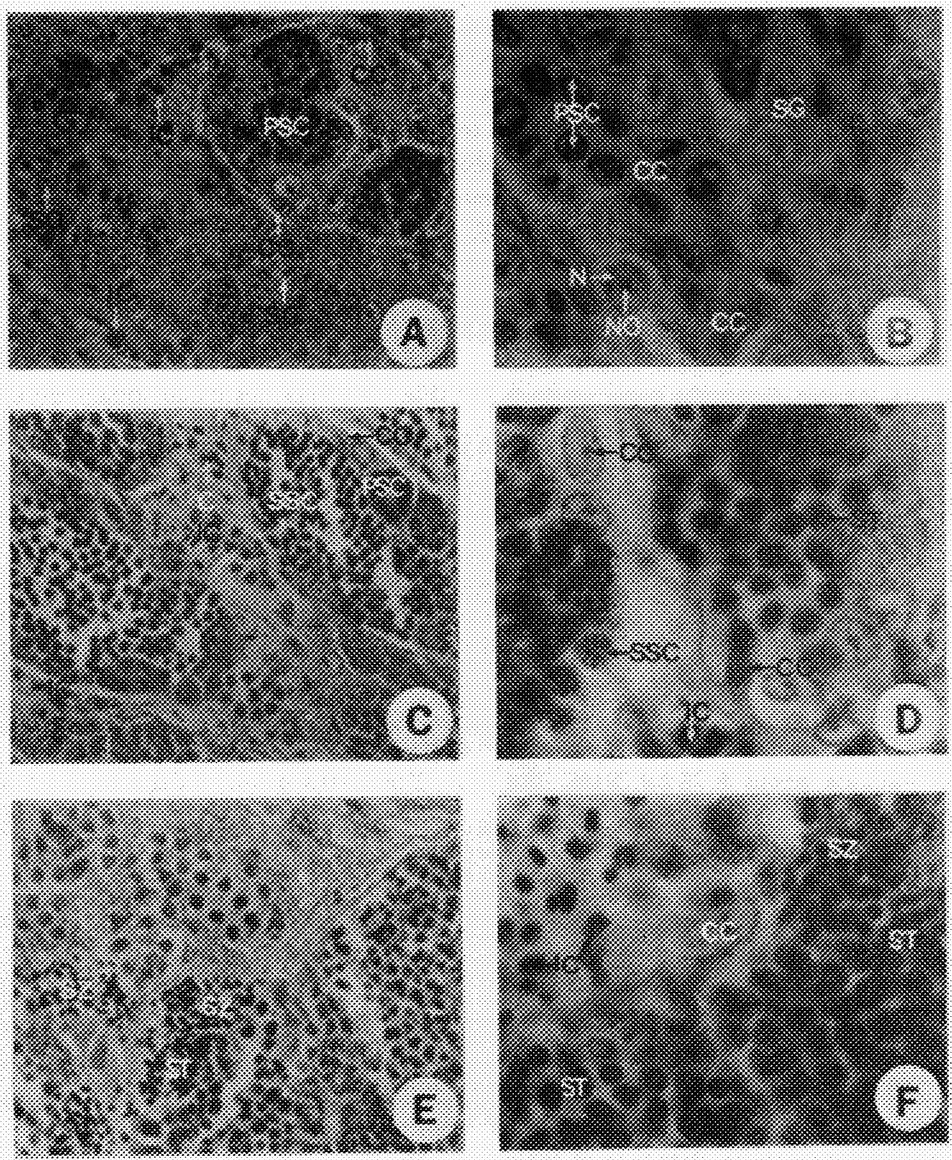

Fig. 4. Light micrographs of the testicular structures during the testicular development (A~F).
A, Section of the early growing testis. Note a number of spermatgonia (SG), the intralobular cyst cells (CC), and a few interstitial cells (IC) in the connective between the lobules; B, Section of the same testis above mentioned. Note a few spermatogonia (SG) with a large nucleus (N) and a remarkable nucleous (NO), and a number of primary spermatocytes (PSC), the intralobular cyst cells (CC); C, Section of the late growing testis. Note the cyst composed of primary spermai~ocytes (PSC), secondary spermatocy~tes (SSC) in the testicular lobules, and a number of the interstitial cells (IC) were well developed around a small capillary (C); D, Section of the testis above mentioned. Note the intralobular cyst cells (CC) were present along the lobule wall.

and secondary spermatocytes(SSC) and spermatids(ST) were shown in the cysts of the testicular lobules, and the interstitial cells(IC) were present in the interlobular spaces; E, Section of a mature testis. Note a great number of spermatids(ST) and spermatozoa are filled in the enlarged testicular lobules; F, Section of the same testis above mentioned. Note a large intralobular cyst cells(CC) and spermatids(ST) on the basement membrane, and several interstitial cells(IC) in the interlobular spaces of the connective tissue; A, C, E : ×800, B, D, F : ×1,500.

With an increase in amount of mature spermatozoa, testicular lobules became larger in size. And in the mature stage, most of the intralobular cyst cells were present along the lobule wall, showed a weak affinity to haematoxylin and became larger in size than those in the late growing stage. Thus, these cells appeared to be much functional during the period of testicular maturation(Fig. 4F).

At this time, the interstitial cells continued to increase in size and their cell area became larger noticeably. Their cell size increased by comparison with previous stages reaching about 8.75±0.20㎛, the nuclei of the cells were 3.90±0.15㎛ in diameter(Fig. 4F).

The fishes collected in June through August had the testes in the ripe and spent stage. At this time, the germinal epithelium was very thin and the cyst were full of spermatozoa. While, some testes showed that the bulk of the spermatozoa had been released from the testicular lobules.

At this stage, the intralobular cyst cells showed some changes in structure of their nucleus, the interstitial cells were still developed. The mean interstitial cell size was 8.40±0.15㎛. The diameter of the cell nucleus was slightly reduced by comparison with the previous stage, the nuclear structure obviously appeared(Figs. 5G, H).

All fishes collected from September to March had the testes in the recovery and resting stage. After spermiation, the undischarged spermatozoa in the lumen of the testicular lobules and the cavity of sperm duct underwent cytolysis and phagocytosis (Fig. 5I). The testicular lobule were contracted and degenerated. Spermatogonia and the intralobular cyst cells on the germinal epithelium were present, especially the intralobular cyst cells showed strong affinity to haematoxylin, while interstitial cells could not be found in this stage(Fig. 5, J).

DISCUSSION

The testes of adult damselfish exhibited seasonal changes in germ cells activities during the reproductive cycle(Lee and Lee, 1987).

In this study, testicular early growth and spermatogenic renewal were initiated from March to April. During this period, primary spermatocytes were observed in many testicular lobules in April, but regions of other lobules still contained only intralobular cyst cells and spermatogonia.

Throghout the year, testicular lobules in *Salmo gairdneri*(Oota et al., 1965) contained resting spermatogonia, which existed singly or in the form of cysts. In this study, the spermatogonia underwent mitotic divisions and proliferated during the early growing stage of the testes. And then, most of the daughter spermatogonia consecutively developed into the sperms, whereas some spermatogonia remained in the resting state at the resting stage as a

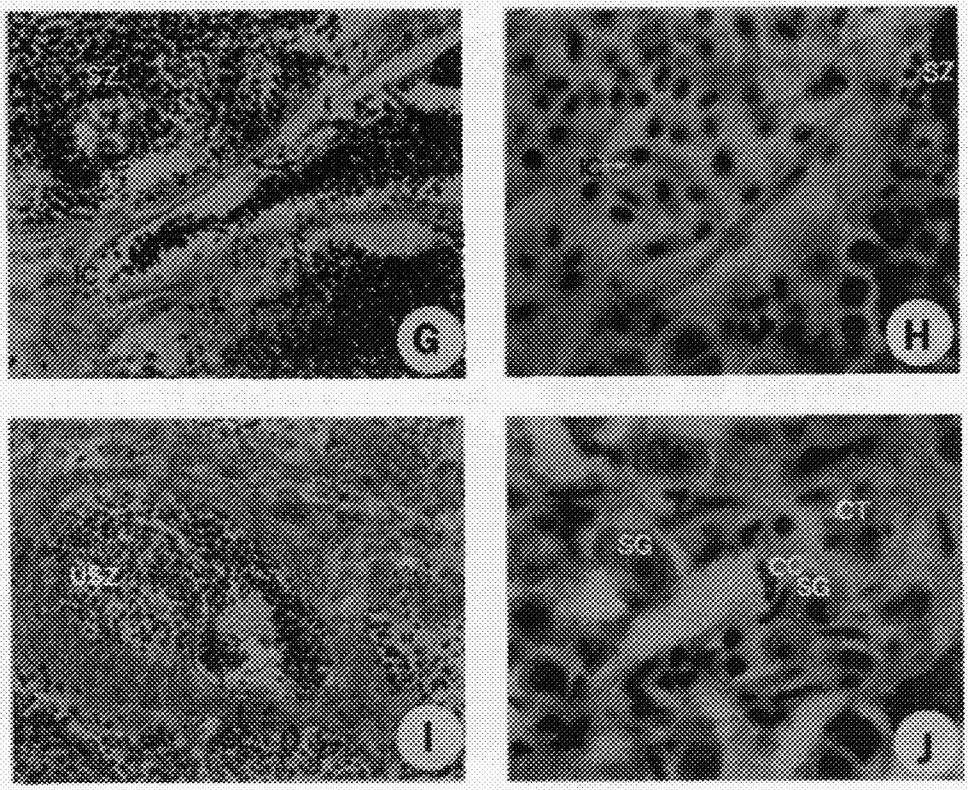

Fig. 5. Light micrographs of the testicular structures duting the reproductive cycle (G~H). G, Section of a ripe and spent testis. Note numerous the interstitial cells (IC) in the interlobular spaces; H, Section of the same testis above mentioned. Note numerous spermatozoa (SZ) and well developed interstitial cells; I, Section of a recovery testis. Note the undischarg-ed spermatozoa (USZ) in the lumen and intralobular cyst cells (CC) in the testicular lobules; J, Section of a testis in the resting stage. Note several remai-ned spermatogonia (SG) and several remakable intralobular cyst cells (CC) were renewed on the connective tissues (CT); G, I: ×600, H, J: ×1,500.

reserver for the next generation of germ cells. Thus, they were shown the same results as described by Oota *et al* (1965).

The most interesting findings in this inverstigation are those which relate to the comparative development of the interstitial tissue elements of damselfish testis. In the early stages, it couldn't be found any histological appearance to be secretory in the interstitial cell, while these cells were spares, and occupied small area. A slight increased in number, size of them occurred during the period of rapid growth. Rapid interstitial cells development occured during the mature and ripe stage. Therefore, it is also suggested that in the early stage of testicular growth, interstitial cells probably dose not mediate pituitary stimulation of spermatogenesis.

On this species, interstitial cells were present but nothing can be said about their secretory nature by the lightmicroscopic study.

In the testis of rainbow trout (Rovertson, 1958) and *Oncorhynchus keta*(Hiroi and Yamamoto, 1968), intralobular cyst cells in the testes showed cytoplasmic vacuolization during the final step of gonadal maturation.

In this species such cytoplasmic vacuolization was not recognized in the intralobular cyst cells during the testicular maturation, while these cells showed a hypertrophic appearance and decreasing affinity in the nucleus to haematoxylin. In spent testes, the intralobular cyst cells became atrophic with an evident decrease in nuclear size. These facts may suggest that the intralobular cyst cells in this species play an important role in testicular maturation extending a certain influence on germ cells as the Sertoli cells in higher vertebrates (Roosen-Runge, 1962; Bloom & Fawcett, 1964).

From our histological observation, it couldn't be conceived with absolute certainty whether exact function of the intralobular cyst cells are homologous with the Sertoli cells in mammals. And they did not show typical features of the steroid producing cells under light microscopy also. Therefore, the cells are not the main steroid producing cells, but they may posses limited metabolic pathways as described by Asahina *et al.* (1983).

The claim of Nicholls and Graham (1972) that in certain teleosts the lobule boundary cell is the Sertoli cell homolog is puzling.

Most of the lobule boundary cell in the teleost is a fibroblastic derivative which lies in the intercellular space and not within the epithelial basement membrane are to be considered as cyst epithelial. Furthermore, since one or more cysts constitute a lobule (Hyder, 1969) and each cyst has its cyst or groups of cysts are surrounded by a connective tissue containing lobule boundary cells (Lofts and Marshall, 1957; Hyder, 1969). In brief, we agree with the statement of Marshall and Loft (1956) and Gresik *et al* (1973) that where lobule boundary cells occur in teleost testes, they are the homolog of the interstitial cells (mammalian Leydig cell), not of the Sertoli cells.

In contrast, those cells described by Nicholls and Graham (1972) which line the lumen of a seminiferous cyst and lie on a basement membrane show a fine structure similar to the report by Gresik *et al* (1973). We feel that these cells are intralobular cyst cells (mammalian Sertoli cells), not lobule boundary cells.

Extensive study is still in progress to elucidate the steroid metabolism in the sepa-rated glandular interstitial cells and intralobular cyst cells of damselfish by the electron microscope study.

要　　約

1991年 2月부터 1991年 9月까지 제주도 서귀포 주변 연안에서 자리망으로 매월 채집된 자리돔 *Chromis notatus*(Temminck et Schlegel) 을 대상으로 精巢發達에 따른 精巢構造와 生殖巢熟度指數의 變化에 關하여 조사하였다. 그 結果는 다음과 같다.

자리돔은 雌雄異體로, 精巢는 좌우 한쌍으로 된 葉狀構造를하고 있으며, 腹腔의 背後部에 척추골을 따라 위치하고 精巢는 左右非相稱이다. 정소의 皮質部는 많은 精巢小葉으로 구성되어 있고, 髓質部에는 各 小葉과 연결된 輸精小管이 있고 이들 輸精小管들은 精巢의 基部에서 輸精管에 합일 연결된다.

本種의 성숙과정은 한개의 cyst내의 모든 세포들은 동시성을 띄며 발달하나, 소엽내 內腔속에 저장되는 정자들은 다소 소엽마다 차이가 있었다. 소수의 정원세포들은 휴지기의 휴지상태로 다음 생식에 관여 하기 위해 잔존 정원세포로 남는다.

정자형성과정은 초기 발달단계에 활발하였으

며, 간질세포의 발달은 이 시기에는 아주 빈약하였다. 간질세포들의 최대 발달은 5월부터 9월까지인 성숙기에 나타나며 분비활성은 뚜렷하게 관찰되지 않았는데, 이는 정소의 초기 성장단계에는 간질세포들이 정자형성을 위해 특별히 뇌하수체의 자극에 관여하지 않는 것으로 추정된다.

소엽내 cyst cell의 활성은 성성숙단계에 따라 뚜렷하게 변했다. 이들 세포들은 얇은 긴타원형 또는 삼각형이며, 이들의 핵과 세포질은 성숙 및 완숙기 중에 크기가 증가된 반면, 이들 세포들은 번식시기 후 헤마톡실린에 강한 친화성을 보였다. 이들 세포들의 분비활성도 광학현미경하에서는 뚜렷하게 관찰되지 않았다.

정소의 발달된 성적단계로 판단해 보면 환경요인들은 정소의 성장과 성숙을 자극하는 것으로 나타났다.

정소의 성숙은 장일주기 및 고수온에서 유발되어 방정기에 이르게 된다. 생식소숙도지수(GSI)는 일장이 길어지기 시작하는 3월부터 증가되지 시작하여, 4월에 점진적으로 증가 되었으며, 고수온기이고 최대 장일주기인 6월과 8월에 최대 값을 나타내어 본종의 정소 발달은 환경요인과 밀접하게 관련되어 있음을 알 수 있다.

REFERENCES

Asahina, k, k. Uematsu and K. Aida. 1983. Structure of the testis of the goby, *Glossogobius Olivaceus*. Bull. Japan. Soc. Sci. Fish., 49(10) : 1493-1498

Bloom, W. and D. W. Fawoott. 1964. A textbook of histology. (pp. 550-583), Philadelphia.

De Wolfe, B. B., and Telford, S. R. 1966. Lipid positive cells in the test is of the lizard, *Cremidophorus tigris*. Copeia., 3 : 590-592.

Gresik, E. W., J. G. Quirk and J. B. Hamilton. 1973. A fine structural and histochemical study of the Leydig cell in the testis of the teleost, *Oryzias latipes* (Cryprindontiformes). Gen. Comp. En-docrinol., 20 : 86-98.

Hiroi, O. and K. Yamamoto, 1968. Studies on the maturation of salmonid fishes-I. Changes in the testis of the chum salmon, *Oncorhyunchus keta*, during anadromous migration. Bull. Fac. Fish. Hokkaido Univ., 19 : 173-184.

Hyder, M. 1969. Hisrological studies on the testis of *Tilapia leucosticta* and other species of the genus Tilapia (Pisces : Tele-ostei). Trans. Amer. Microsc. Soc. 88 : 211-231.

Lee, Y. D and T. Y. Lee. 1987. Studies on the reproductive cycle of damselfish, *Chromis notatus*(Temminck et Schlegel). Bull. Korean Fish. Soc., 20(6) : 509-519.

Lofts, B. and A. J. Marshall. 1957. The Leydigcell homologues in certain teleost fishes. Nature., 177 : 704-705.

Lofts, B. 1972. The sertoli cell. Gen. Comp. Endocrinol., Suppl., 3 : 636-648.

Marshall, A. J. and Lofts, B. 1956. The Leydig cell homologue in certain teleost fishes. Nature(London)., 177 : 704-705.

Marshall, A. J. 1960. Reproduction in male bony fish. Zool. Soc. London Symp., 1 : 137-150.

Nicholls, T. J. and G. P. Graham. 1972. The ultrastructure of lobul boundary cells and Leydig cell homologs in the testis of a cichlid fish, *Cichlasoma nigrofasciatum*. Gen. Comp. Endocrinol. 19 : 133-146.

Oota, I., Yamamoto, K., K. Takano, and T. Sakaguchi. 1965. Studies on the maturing process of the rainbow trout, *Salmogairdneri*. Maturation of the testis of a one-year old fish. Bull. Jap. Soc. Sci. Fish., 31 : 597-605(In Japanese with English Summary).

Rovertson, O. H. 1958. Accelerated

development of testis after unilateral gonadectomy, with observations of the normal testes of rainbow trout. Fish. Bull., 127 : 9-30.

Roosen-Runge, E. C. 1962. The process of spermatogenesis in mammals. Biol. Rev., 37 : 343-377.

Russell, L. D., H. P. Ren, I. S. Hikim, W. Schulze and A. P. S. Hikim. 1990. A comparative study in twelve mammalian species of volume densities, volumes, and numerical densities of selected testis components, emphasizing those related to the Sertoli cell. American J. Anat., 188 : 21-30.

Sathyanesan, A. G. 1959. Seasonal histological changes in the testes of catfish Mystus seenghala (Sykes). Jour. Zool. Soc. India., X I, 52-59.

Stanley, H. G. Chieffi and V. Botte. 1965. Histological and histochemical observations on the testis of *Gobius paganellus* Z. Zell-forsch., 65 : 350-362.