Northwest Atlantic Fisheries Organization

Serial No. N518

NAFO SCR Doc. 82/VI/30

SCIENTIFIC COUNCIL MEETING - JUNE 1982

Some Histological Observations on Gonadal Development of *Illex illecebrosus* (Le Sueur)

by

M. L. Coelho

Instituto Nacional De Investigacao das Pescas
1400 Lisboa, Portugal

and

T. Amaratunga

Fisheries Research Branch, Department of Fisheries and Oceans
P. O. Box 550, Halifax, N.S. B3J 2S7

and

H. Dupouy

Institut Scientifique et Technique des Pêches Maritimes
B. P. 1240, Saint-Pierre et Miquelon - F-97500

INTRODUCTION

Visual morphological criteria have been standardized (Amaratunga and Durward, 1978) and often used to describe maturity conditions of Illex illecebrosus (Mercer, 1973; Amaratunga, 1980). While this staging presents satisfactory descriptions of squid for routine biological sampling, the method does not provide an indication of the progression of gametogenesis in the gonads. Histological studies are required to describe these processes. While studies on gonadal tissue have been reported for other cephalopods such as Sepia officinalis (Richard, 1971) and many Loliginidae species, data on I. illecebrosus are limited.

In this paper a histological approach is taken to describe cellular structure and development of the maturing process of I. illecebrosus. The processes are studied in a wide range of animals and the chief aim is to discuss phases observed by histological methods and relate them to the visual criteria methods currently used in field work.

MATERIAL AND METHODS

Two specimens were collected from each available 10 mm size class for both males and females. Specimens available from adult size classes, ranged from 155-230 mm in males, 205-280 in females respectively.

Samples were collected during the R.V. Thalassa cruise (August 28 - September 21, 1981) conducted on the Scotian Shelf, using a bottom trawl. Freshly caught squid were first analyzed for standard morphometrics (Amaratunga and Durward, 1979). Gonadal observations were obtained by excising ventrally to expose ovaries and testes. The morphometric observations of the gonads included length of testes, widest width of testes, condition of spermatophore development (visual), length of ovary, widest width of ovary, condition of ovary and oviduct.

Two sections, each about 5 mm thick, were taken at section I and II (Fig. 1) and immediately fixed in seawater Bouin's.

Clearing and embedding were carried out using standard histological techniques. We used paraffin to embed and a Leitz 1207 Microtome to section at 5μ thickness. Stain used was hematoxilin/esosin. Nikon biological microscope OPTIPHOT was used and photomicrographs were taken. Oocytes and spermatocytes were studied using a 10x ocular micrometer. Measurements taken on all gamete cells were at the largest diameter.

RESULTS

Observations were made on 18 slides from males and 20 slides from females. Gamete cell developmental patterns showed that a range of gonadal development existed in the size range of squid studied. The developmental processes were distinguished at six steps, similar to the proposed scheme for Sepia officinalis by Richard (1971). The steps listed in Figure 2 provided us with a means of categorizing the development process observed in this study.

According to the largest diameter measurements taken of the cells (Fig. 3) and the observable shape and structure of cell differentiation (PL 1,2,3), the gonads studied were further categorized as follows:

Females - Two groups of maturing categories were found.

Group A - Consisted of mature but relatively small females with mantle lengths ranging from 205-210 mm. In this group we could not observe any germinal cells (oogonia) nor early oocytes (steps 1,2,3). Only late oocytes (steps, 4,5,6) were frequently found, with the predominant cells in stage 5 and 6 (Plate 3F). The average maximum diameter was about 600 μ. Steps with typical advanced follicular development as well as advanced cell size and differentiation could easily be observed. Eccentric nuclei were also well distinguished (Plate 3E).

Group B - Consisted of maturing females with mantle lengths range from 215-280 mm. In this group we could observe, near the alveolar walls, a few germinal cells of diameter 15-25 μ (step 1). These were intensively stained and were probably developing oogonial cells. From the periphery to the inner-most part of alveoli we observed cells with different growth and development forms. First, 40-70 μ oocytes I showing a small cytoplasmic area in relation to nucleus size (step 1-2). Second, larger cells of 79-80 μ diameter (step 2-3), with a larger cytoplasmic area. Around these cells dispersed follicular cells were evident. Cells with most advanced follicular development (115-173 μ) were most common in this group, step 3-4. We also noticed that females with 270-280 mm in mantle length were the most advanced, predominantly containing steps 3, 4 while cells in stage 1-2 were rarely found (Plate 3 - A to D).

No significant difference between cellular tissue condition from section I and section II was noticed.

Males

Group I - Mantle lengths ranging from 155-170 mm. Gonadal tissue having a homogeneous structure corresponding to steps 1-2. Alveoli show characteristic polygonal formation (PL 1 + 2A). Within alveoli all sizes of cells were present although the predominant ones were 7-9μ and some of these were attached to the alveolar walls. Other cells ranging from 5-7μ were present in the inner part of the alveoli. We noticed a typical shape differentiation from ovoid/stalked cells near the walls to spherical and free cells inside the lumen (PL 1B). This is characteristic of primary spermatocyte differentiation. No spermatids were noticed.

Group II - Consisted of a more developed maturing condition but these were not easily separable at the optical level used. In this group mantle lengths range from 200-300 mm (step 2-5). The predominant cells were spermatocytes II (with cell sizes 5-7μ) and spermatids (cells 1-4μ). The spermatids present were at maturing and mature conditions, having characteristic spherical and flat shapes respectively. The maturing spermatids became more common in the larger males and gonadal tissues development had progressed to the most advanced stages in correspondence to animal sizes from 225-230 mm. The most advanced ones had the alveolar lumen filled with mature spermatids (steps 4, 5).

Separation of slides from section I and II was not clearly evident.

DISCUSSION

Figure 4 summarizes all data obtained in this study. Morphometrics using visual criteria to establish maturity condition (Durward et al., 1978) and gonadal tissue development steps from histological observations are listed.

As we only had access to a small part of the Illex life cycle (September, 1981) the main intention was to compare the macroscopic observations made by standard visual biological sampling with the corresponding microscopic cellular conditions. In spite of the restricted number of samples obtained, we were able to conclude that results obtained by visual criteria closely correspond to the microscopic observation in the same animals. This was particularly true in females as demonstrated in Figure 4 where females from 215-280 mm showed visual maturity stages corresponding closely to the histological steps. As a confirmation, we found females no. 13 and 23 had the same mantle length but different histological maturity stages, corresponding closely with the maturity indexes calculated by Durward *et al.* (1978) method of gonadal to body length ratio (NGL/LM). Individual No. 23 had a larger nidamental gland (NGL) than NGL of No. 13. This one was also greater in total weight. These observations validate the possibility of categorizing the visual criteria and relating it to the histological maturing process in females.

There were more discrepancies between maturity stages by visual criteria and the histological steps observed in males. According to our results from histological observations (Fig. 4), animals No. 14 and 15 should be classified as maturing stage 2 rather than as maturing stage 1 as indicated by visual criteria. The total weight of these examples as well as testes length are considerably higher than in all others labelled as visual stage I. Histologically it also became apparent that the production of spermatozoa is a continuous and gradual process; the spermatophoric sac permits the accumulation of sperm bundles

of spermatophores within it. The spermatophores may then be released at any subsequent time. The histological steps observed show that spermatophores may be continually formed after the males reached a late development stage. This is not apparent in the visual method although morphological changes were described in stages by Durward et al., (1978). Particularly in the early stages of male maturity, the morphological characteristics are less sensitive to actual gonadal development as seen by histological observations.

On the other hand the maturing process in advanced females seems to take a considerably shorter period of time. This was demonstrated by our observation that mature females (Step 5-6) carried over 90% of late oocytes. This fact can also be correlated with the production of the spawning egg masses during a restricted period of time.

In conclusion we find that the visual criteria correspond reasonably well with the histological observations. However, in view of the disparities noted in this study it is suggested that further histological studies will permit us to better interpret the gonadal condition described by visual criteria.

REFERENCES

Amaratunga, T. and R.D. Durward. Field Guide for data collection for the squid Illex illecebrosus - ICNAF Res. Doc. 78/11/5.

Amaratunga, T. 1980. Growth and maturation patterns of the short-finned squid (I. illecebrosus/on the Scotian Shelf. NAFO Scr. Doc. 80/II/30. Serial No. 62.

Botlke, Werner. 1974. The fine structure of the ovarian follicle of Alloteuthis subulata Lam. (Mollusca Cephalopoda) Cell Tissue Res., 150: 463-479.

Durward, R.D., T. Amaratunga, and R.K. O'Dor. 1978. Maturation index and fecundity for females Illex illecebrosus (Le Suer, 1821). ICNAF Research Bulletin No. 14, 1979.

Grieh Thomas M. and Robert D. Buman. A study of spermatogenesis in the spawning population of the squid, Loligo opalescens Fish Bulletin, 169. pg 11-22. California.

Mercer, M.C. 1973. Sexual maturity and sex ratio of the Ommastrephidae squid I. illecebrosus (Le Sueur, 1821), at Newfoundland (Subarea 3). ICNAF Res. Doc. 75/71, Serial No. 3023.

Mesnil, B. 1976. Growth and life cycle of squid, Loligo pealei and I. illecebrosus from Northwest Atlantic. ICNAF Res. Doc. 76/VI/65.

Richard, A. 1971. "Contribution à l'étude experimentale de la croissance et de la maturation sexuelle de Sepia officinalis. L." Université de Lille. France.

Recksiek, W. Conrad and W. Hubert. 1978. Histological observation on oogenesis in Loligo opalescens. Fish. Bulletin 169 pg 23-33. California.

Takahashi, N. and Takehiro Yahata. 1973. Histological studies on the maturation of the ovary in the squid, Todarodes pacificus. Bull. Fac. Fish. Hokkaido Univ. 24: 63-68.

Yung-Ko, Ching. 1930. Contribution à l'étude cytologique de l'ovogenese du development et de quelques organes chez les cephalopodes. Ann. Inst. Oceanogr. (Monaco), 7: 301-364.

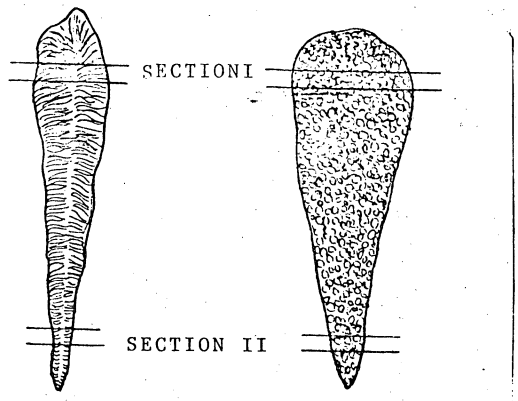


Fig. 1. Locations where tissues samples were taken from testes and ovaries.

	Females	step	Males
	Oogonia - Germinal cells	1	Spermatogonia - Germinal cells
OOCI	Growth - Yolkless stage	2	Spermatocyte I
OOCI	Growth - Early yolk stage	3	Spermatocyte II
OOCI	Follicle cells development	4	Round spermatid
OOCI	Follicle cells decrease	5	Flat spermatid
OOCII	Mature oocyte	6	Spermatozoa

Fig. 2 - Phases of cellular development adapted to Illex illecebrosus maturing gonadal tissue.

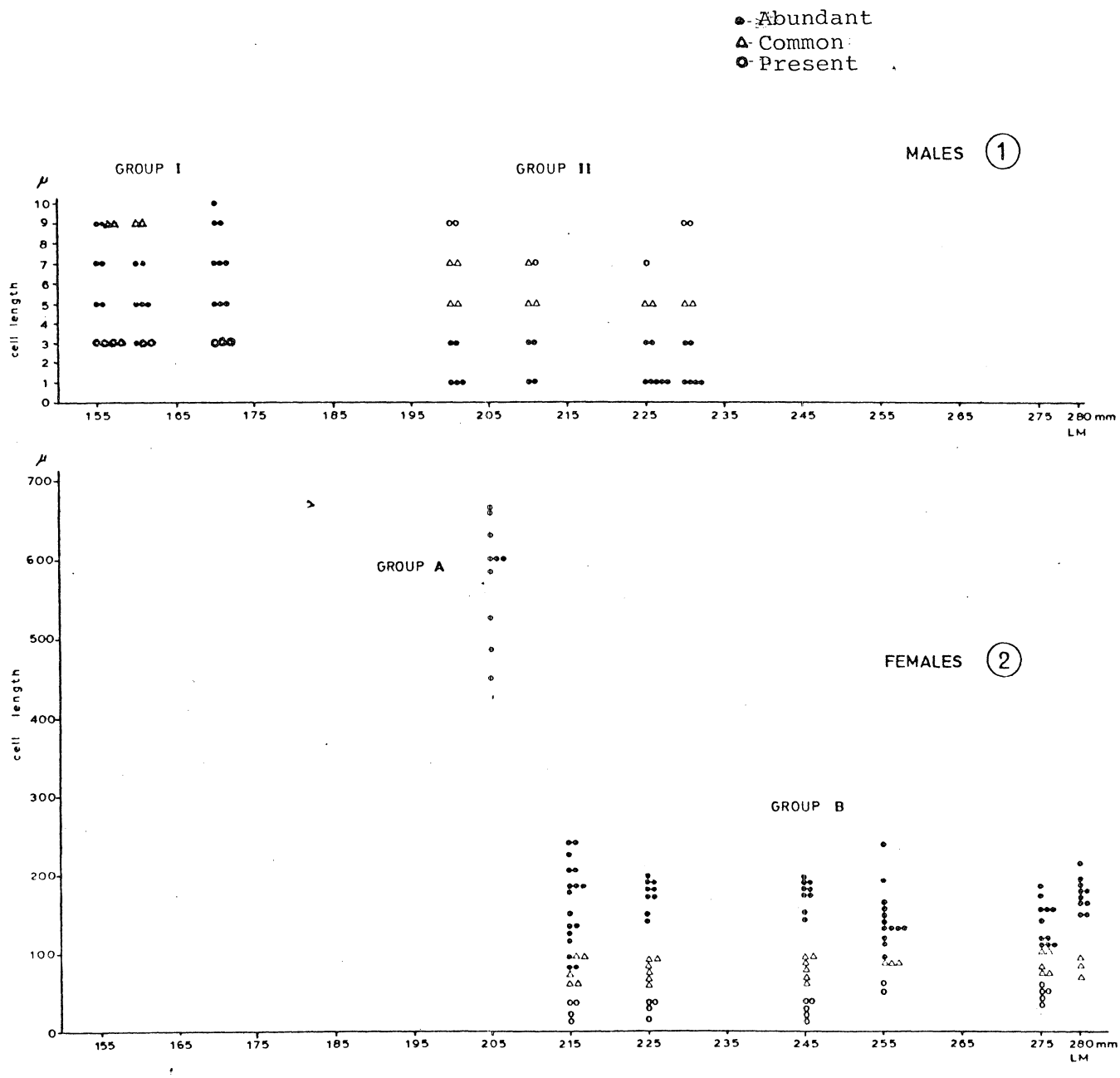


Fig. 3. Measurements (largest diameter in μ) of gonadal cell tissue, of males (1) and females (2)

MALES

STATION Nº	EXAM. Nº	LENGTH MANTLE(LM) mm	TOTAL WEIGHT(W) g	TESTIS LENGTH/WID. mm	MATURITY STAGES (I-IV) a) (VIS. CRITERIA)	HISTOLOGICAL CONDITION (STEP 1-6)
166	27	155	80	32/ -	I	MATURING 1-2
166	26	160	100	35/ -	I	MATURING 1-2
176	16	170	100	41/5	I	MATURING 1-2
154	14	200	180	68/19	I	MATURING 2-3
154	15	200	170	55/12	I	MATURING 2-3
154	3	210	210	76/20	II	MATURING 2-3
154	5	210	180	75/18	II	MATURING 2-3-4
154	9	225	240	92/26	II	MATURING 2-3-4-5
154	2	230	290	94/26	II	MATURING 2-3-4-5

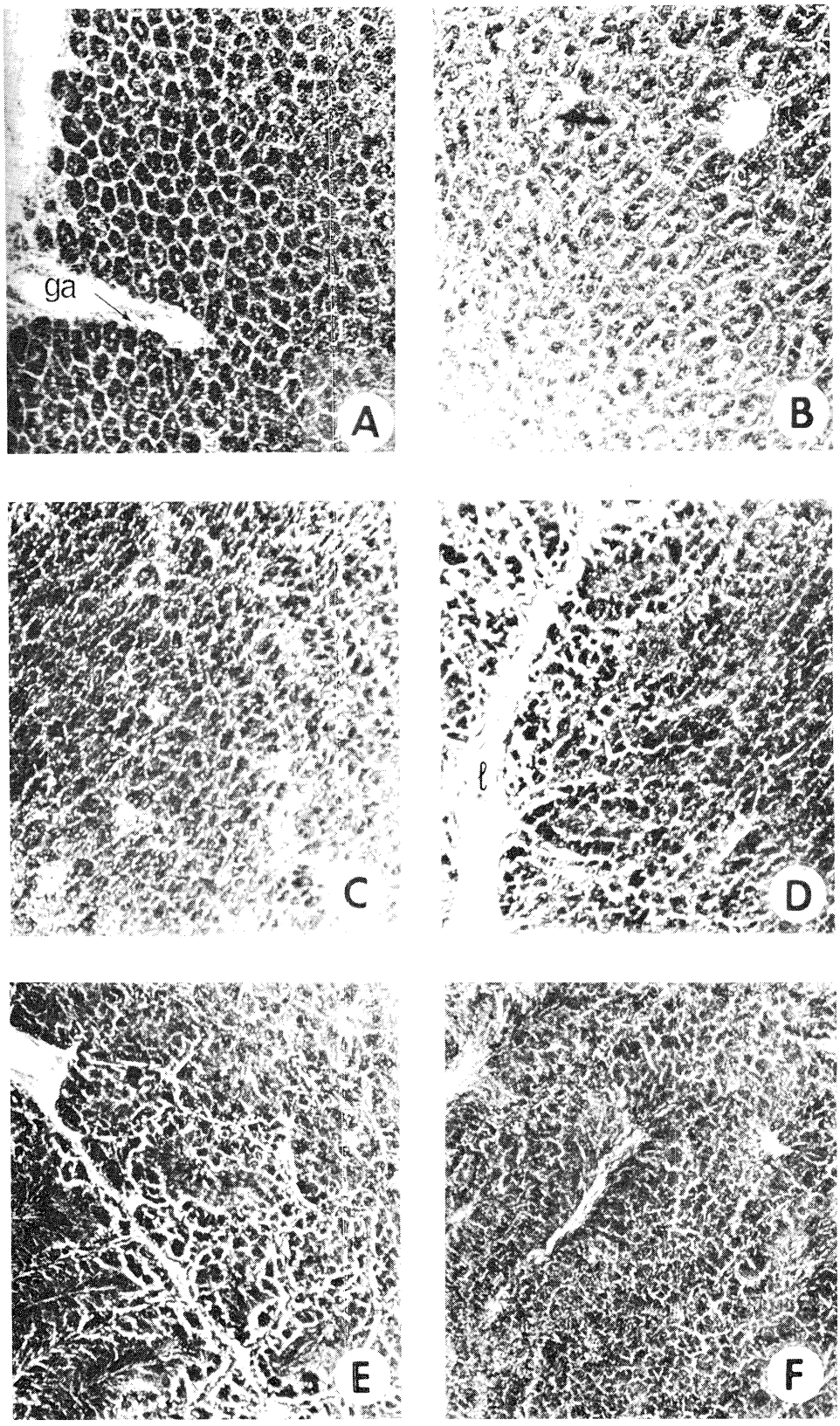
FEMALES

STATION Nº	EXAM. Nº	LENGTH MANTLE(LM) mm	TOTAL WEIGHT(W) g	NGL mm	OVARY LENG/WID. mm	MATURITY INDEX (VIS.CRIT.)	CORRES. MATUR. STAGE (I-V) b)	HISTOLOGICAL (STEP 1-6) CONDITION
84	-	206		95	—	0.46	V	MATURE 5-6
196	1	210	170	80	—	0.38	V	MATURE 5-6
154	13	215	190	22	66/13	0.10	I/II	MATURING 2-3
160	23	215	250	41	58/15	0.19	III	MATURING 3-4
154	8	225	280	30	68/18	0.13	III	MATURING 3
160	12	230	250	34	52/13	0.14	III	MATURING 3
154	11	245	320	38	77/18	0.15	III	MATURING 3
154	1	256	260	34	74/14	0.13	III	MATURING 3
160	33	275	460	46	58/15	0.17	III	MATURING 3-4
160	18	280	420	44	78/16	0.16	III	MATURING 3-4

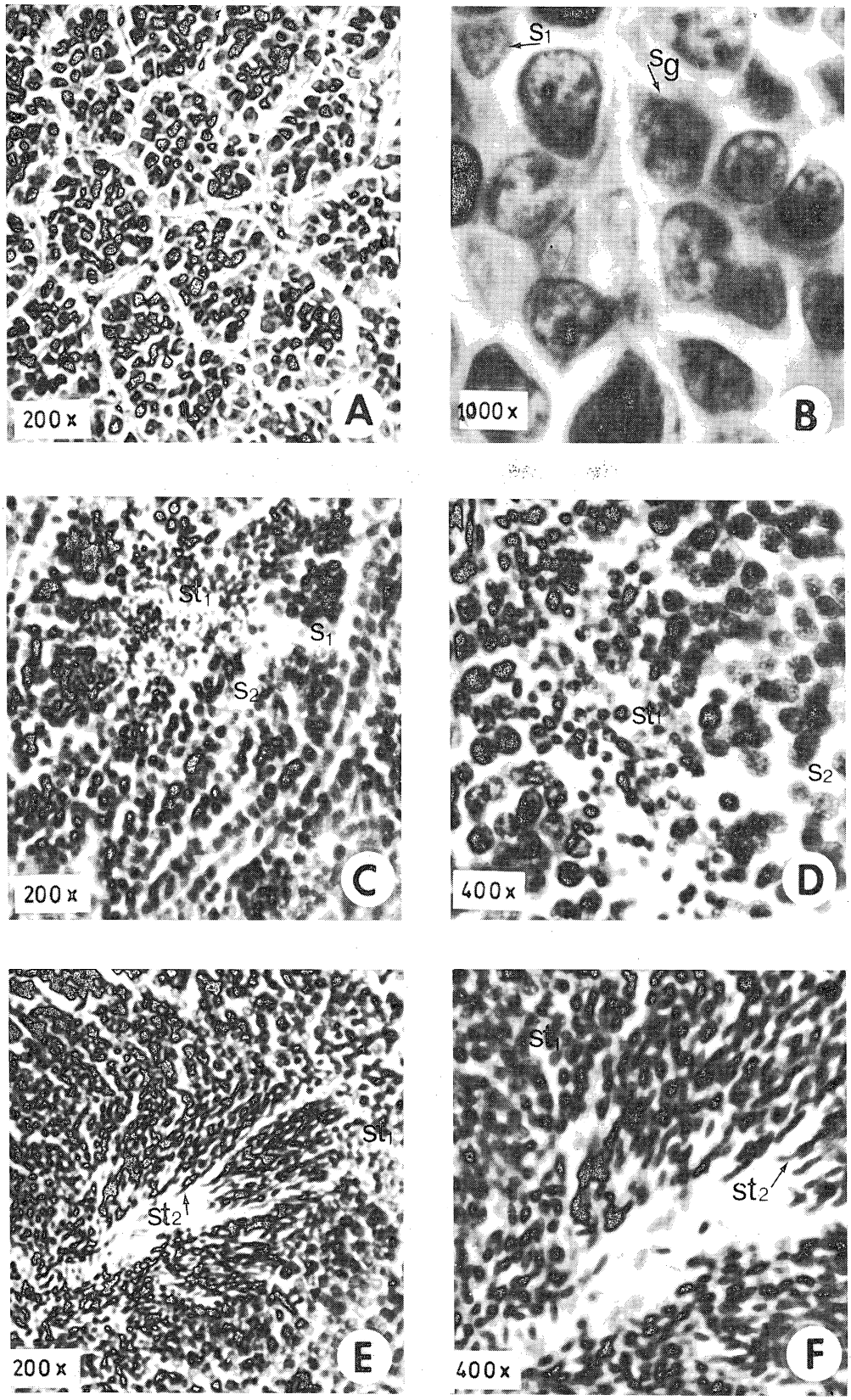
FIG 4 - MORPHOMETRICS DATA. HISTOLOGICAL STEPS (1-6) ADAPTED IN THIS STUDY COMPARED WITH MATURITY STAGES PROVIDED BY MACROSCOPICAL CRITERIA.

a)-Maturation stages - MALES - I-IV
Amaratunga and Durward, 1978

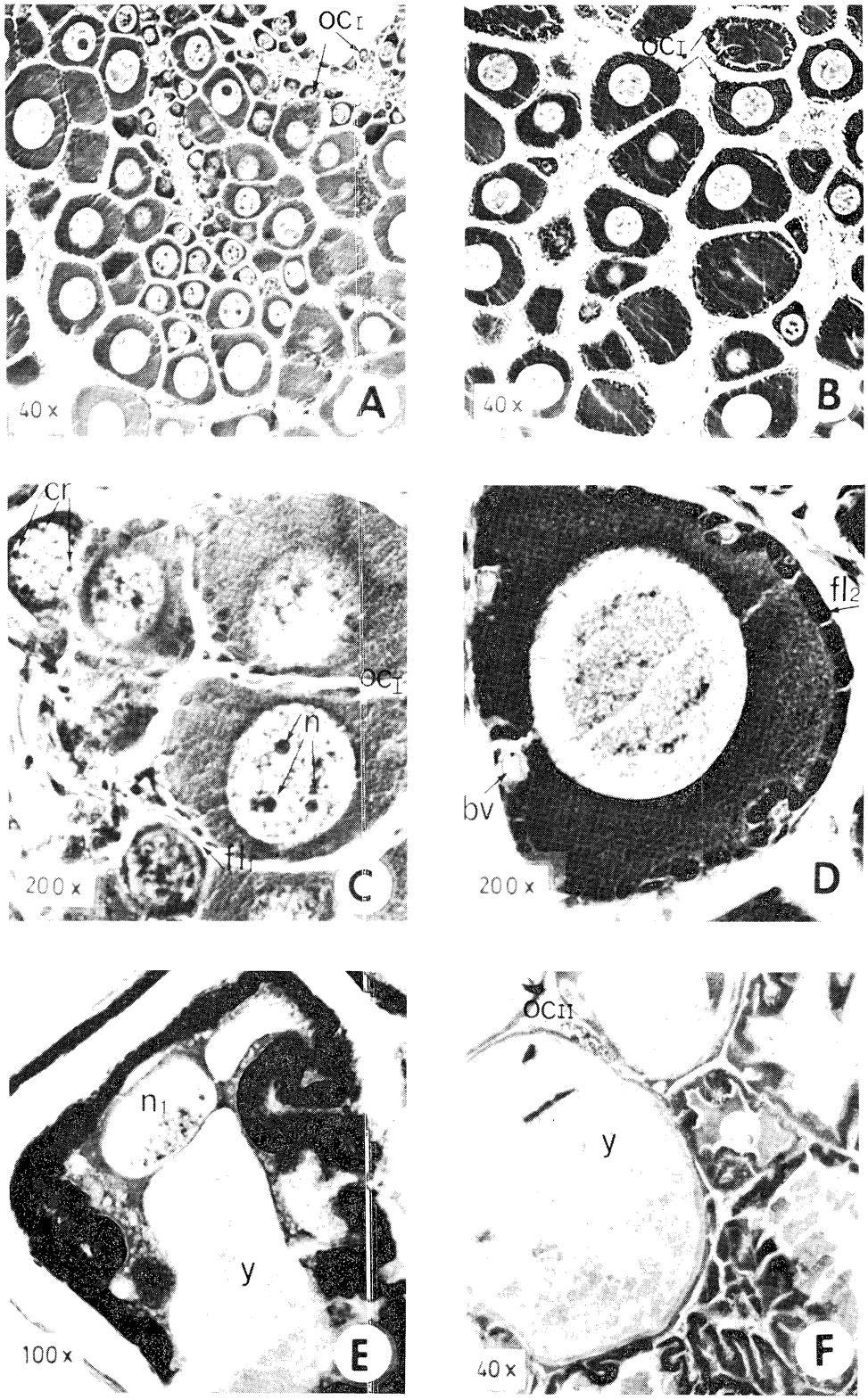
b)-Maturation stages - FEMALES - I-V
Durward, Amaratunga and O'Dor, 1978



Pl.1-Testicular tissue.40x. A and B- LM 160-170 mm- maturing 1-2; C and D- LM 200-210 mm- maturing 2-3; E and F- LM 225-230 mm maturing 3-4-5;ga:genital aorta;l:longitudinal lumen.



Pl.2-Cell differentiation from spermatogonia to mature spermatids. A-general aspect of alveoli;B-sg:spermatogonia;s_1: spermatocyteI: C and D-s_2:spermatocyteII;st_1:immature spermatids; E and F-st_2: mature spermatids.



Pl.3-Ovary tissue. A and B-oocytesI in various maturation stages - LM 205-210 mm. C to F-ocI:oocytesI;fl_1:flat follicle cells;n: nucleoli; cr:chromatin;bv:blood vessel; fl_2:cuboidal follicle cells showing initial penetration;n_1:nucleus;y:yolk; F-ocII; oocytesII.LM 215-280 mm.