CHAPTER IV

RESULTS

Macroscopic Examination

1 Sex Ratio

A total of 1,911 *E. heteroloba* consisting of 696 females and 1,215 males were collected for macroscopic examination. Total lengths of the females sampled were 5.0-9.1 cm (mean±SD = 7.43±0.74); those of males were 4.9-9.1 (mean±SD = 7.29±0.79). Males were dominant in almost all sampling periods (Figure 3). The overall ratio of females to males was 1:1.75 which was significantly different from the hypothetical 1:1 ($\chi^2 = 92.59$, d.f.= 23, P< 0.01).

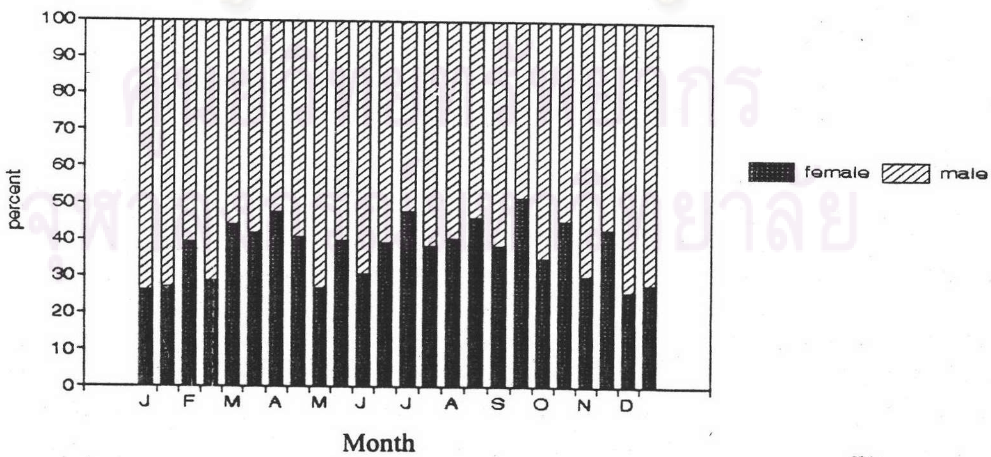

Figure 3. Proportion of female to male shorthead anchovy sampled in 1995

2. <u>Batch Fecundity</u>

Batch fecundity was estimated from 155 mature females of total lengths ranging from 5.4 to 9.2 cm. Any lobe or any portion within lobe did not affected the estimation of batch fecundity (P> 0.05). The number varied from 101 to 4,606 yolked oocytes per fish. As the pooled data scattered unevenly (Figure 4), total lengths were grouped into 9 classes with 0.5 cm interval before fecundity was calculated for each class interval. The linear relationship was expressed as

$$\log F = 2.09 + 1.21 \log L \quad (r^2 = 0.68, P<0.001)$$

where F is batch fecundity and L is the midpoint of 0.5 cm length class

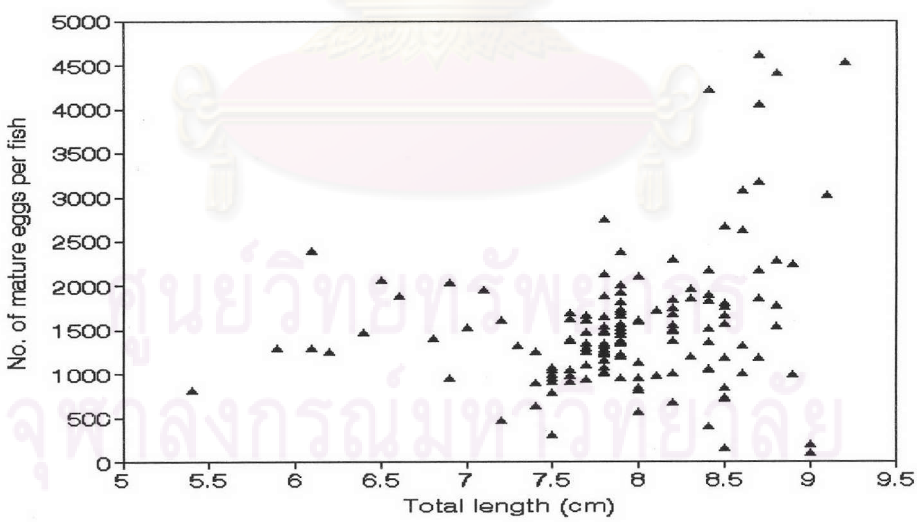

Figure 4. Scatter diagram showing the relationship between total length and batch fecundity of shorthead anchovy

3. Gonadosomatic Index

For all sampling periods, gonadosomatic index of female *E. heteroloba* of mean total lengths ranging from 6.5 to 8.5 cm were 0.87- 4.16. Those of males of total lengths ranging from 5.8 - 8.3 cm were 1.52 - 5.72. GSI for both sexes showed a similar trend, although the index of females was considerably lower than that of males (Figure 5). This indicated that the timing of ovarian development coincided with that of testis development. GSI values were high at the beginning of the year from January to April following by a decline from May to September, then increased again from late September to the end of the year with moderately high values. Fluctuation was found within each month.

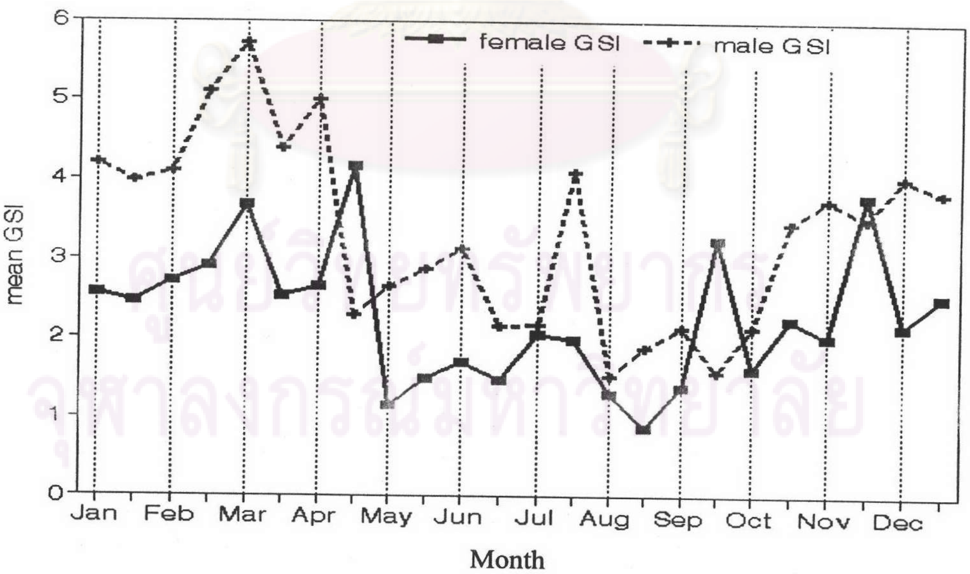

Figure 5. Female and male gonadosomatic indice of shorthead anchovy during 1995

4. Gonad Development

Macroscopic examination of maturity stages indicated that high proportion of mature fish was obtained during late October to early March which corresponded to the Northeast Monsoon period in this area. Males were found to be more mature than females; however, macroscopic results showed same trends of maturity in females and males (Figure 6). Yearly variation of mature fish seemed to coincide with GSI in both females and males (Figures 7& 8)

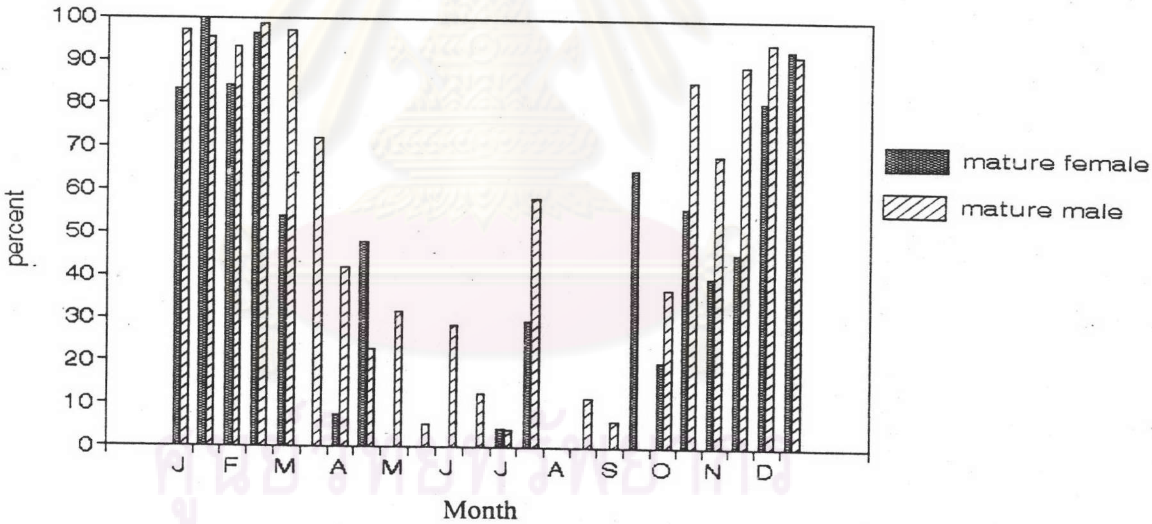

Figure 6. Percent of mature female and male shorthead anchovy sampled during 1995

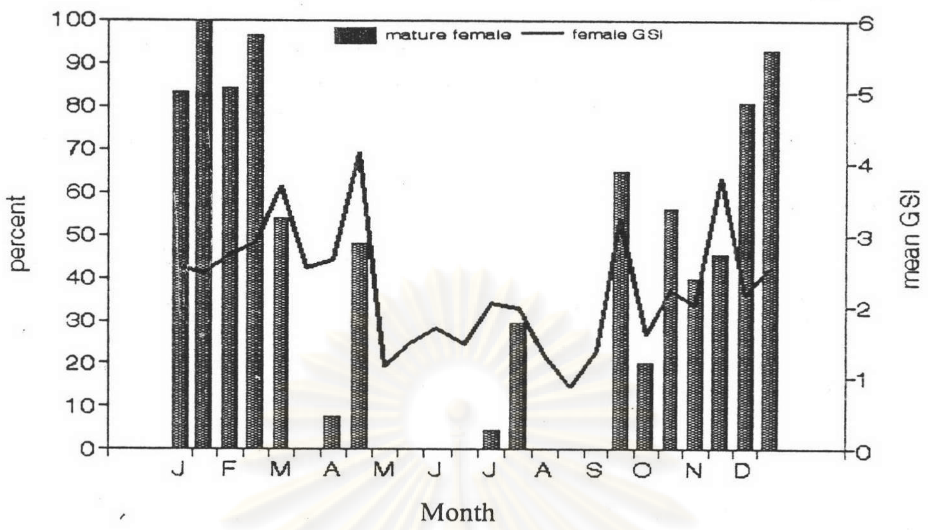

Figure 7. Percent of mature females shorthead anchovy and their mean gonadosomatic indice during 1995

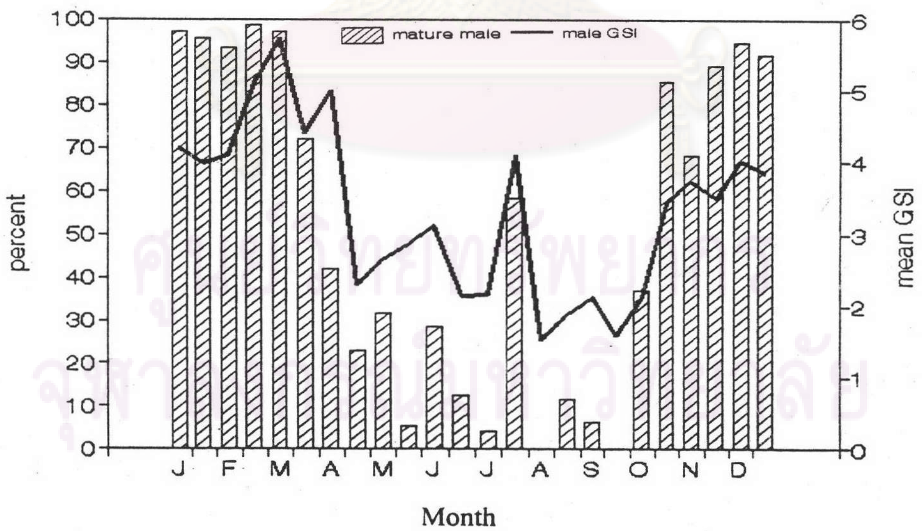

Figure 8. Percent of mature males shorthead anchovy and their mean gonadosomatic indice during 1995

Microscopic Examination

1. Stages of Oocyte Development

The oocyte development of *E. heteroloba* were classified histologically into 4 stages related to those of macroscopic classification: immature, developing, mature, and ripe oocytes. All stages except ripe oocytes were found in this study. The characteristics for each oocyte stage of *E. heteroloba* were as followed:

1.1 Immature Oocytes (Figure 9)

These primary oocytes were in perinucleolar stage. Cytoplasm was seen as narrow and strongly basophilic zone which stained well with haematoxylin around the weakly basophilic nucleus. Oocytes in early perinucleolar stage were small and rather round shape with a comparatively large nucleus contained several nucleoli located periphery. Late perinucleolar oocytes were more ovoid and the nucleoli became flattened against nuclear membrane. Follicular epithelium was not well developed at this stage showing very thin layer.

1.2 Developing Oocytes (Figure 10)

These oocytes were in the primary yolk vesicle stage, characterized by the formation and accumulation of yolk. The cytoplasm lost some of its basophilic property. The innermost zone was still more compact and more basophilic stained, while zone of small protein yolk vesicles appeared in the outer cortex. Oil globules were accumulated periphery. They appeared in empty vacuoles due to their dissolution during histological process. The outline of the nucleus became irregular and the flatten nucleoi were in close contact with cytoplasm. Zona radiata developed between cytoplasm and follicles, stained pink layer in the section.

1.3 Mature Oocytes (Figure 11)

Oocytes were in the late yolk vesicle stage. Zona radiata was clearly visible and almost all cytoplasm was filled with acidophilic lipid yolk vesicles, stained pink colour. The columnar follicle epithelium was more prominent. Nucleus appeared roughened and indistinct. Only a small part of the original compact cytoplasm remained.

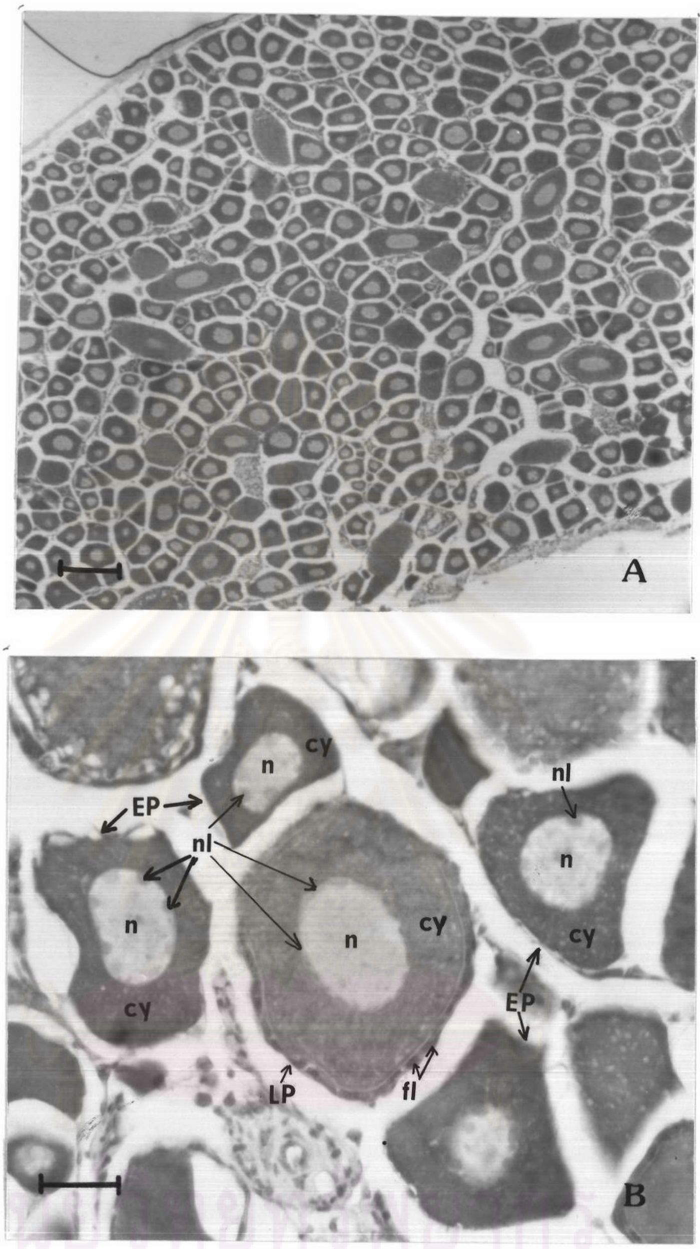

Figure 9. Immature oocytes in the ovarian sections of *E. heteroloba*

n: nucleus, nl: nucleolus, cy: cytoplasm, fl: follicular cell

EP: early perinucleolar stage oocyte, LP: late perinucleolar stage oocyte

(Buffered Formalin; Haematoxylin and Eosin)

Bar scale: A = 100 μm, B = 20 μm

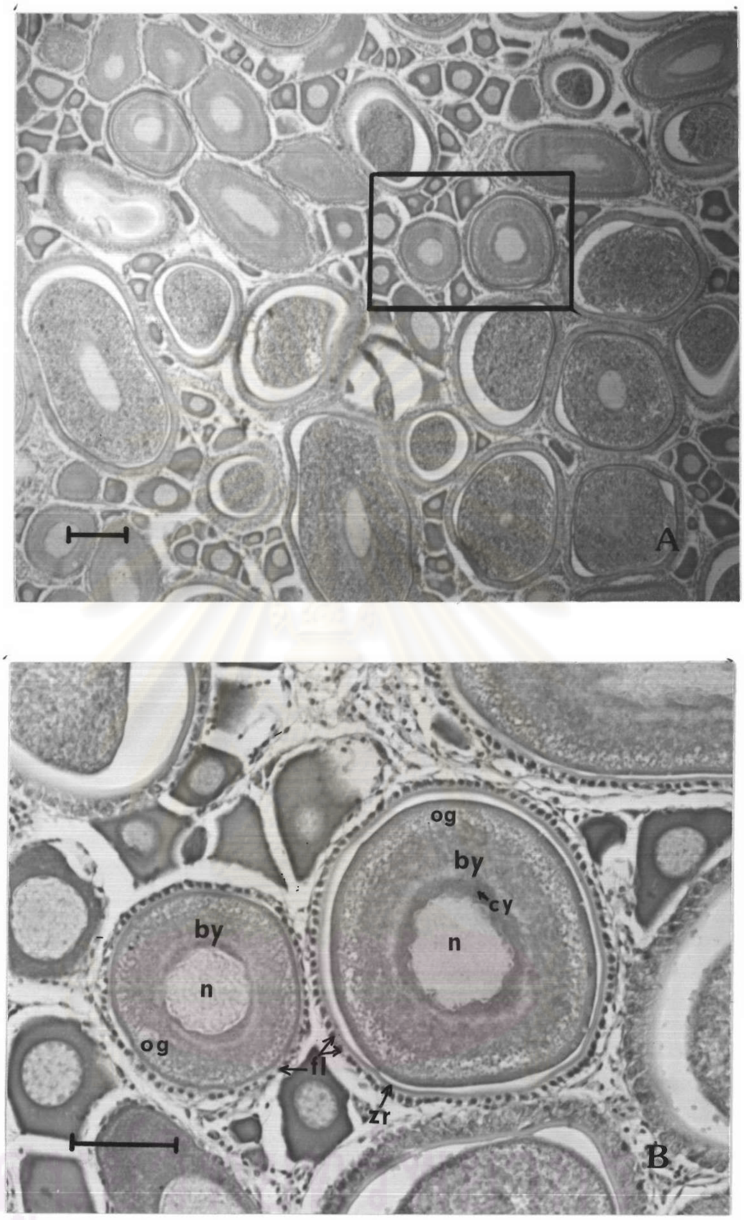

Figure 10. Developing oocytes in the ovarian sections of *E. heteroloba*

n: nucleus, og: oil globules, by: basophilic yolk vesicles, zr: zonar radiata, fl: follicular cell

(Buffered Formalin; Haematoxylin and Eosin)

Bar scale: A = 100 μm, B = 50 μm

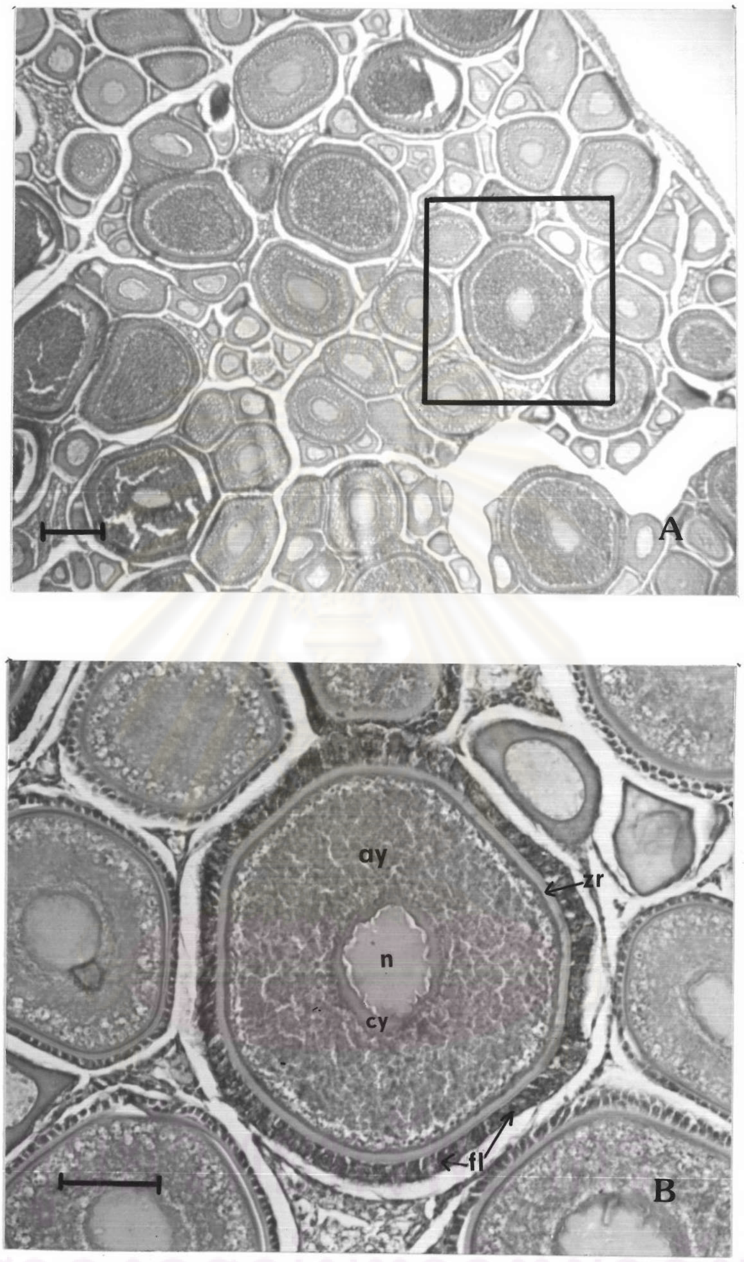

Figure 11. Mature oocytes in the ovarian sections of *E. heteroloba*

n: nucleus, ay: acidophilic yolk vesicles, zr: zonar radiata, fl: follicular cell

(Buffered Formalin; Haematoxylin and Eosin)

Bar scale: A = 100 μm, B = 50 μm

2. Incidence of Postovulatory Follicles

Postovulatory follicles (Figure 12) commonly found in the mature ovaries of *E. heteroloba* were new postovulatory follicles as described for northern anchovy by Hunter and Macewicz (1985). They were irregular in shape with an irregular lumen. The granulosa epithelial cells were columnar or cuboidal with prominent nuclei and slightly hypertrophy collapsing into the lumen. The nucleus of granulosa cells located at either the apex or the base of the cell. The underlying thecal cells appeared loosely attached to the granulosa cell layer. Postovulatory follicles were found scattering in the ovaries throughout the year. However, no fully spent ovary filled entirely with postovulatory follicles were found.

2. Incidence of Artetic Oocytes

The atretic oocytes of *E. heteroloba* (Figure 13) were characterized by proliferation of granulosa follicular cells and broken down zonar radiata. Yolk vesicles and nucleus were fused. Atretic ocytes appeared commonly during the sampling periods with high number around late April to early August (Figure 14).

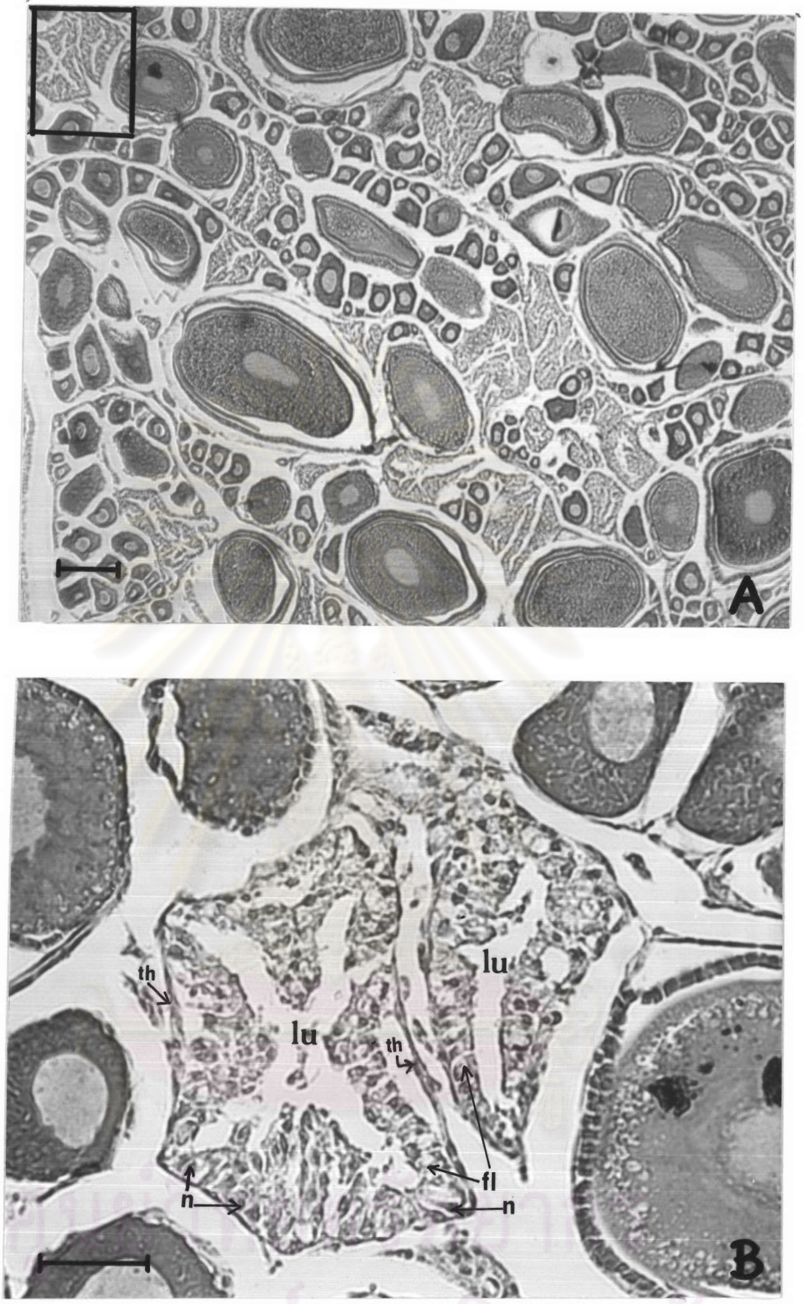

Figure 12. Postovulatory follicles in the ovarian sections of *E. heteroloba*

n: nucleus of follicular cell, fl: follicular cell, lu: lumen, th: thecal cell

(Buffered Formalin; Haematoxylin and Eosin)

Bar scale: A = 100 μm, B = 50 μm

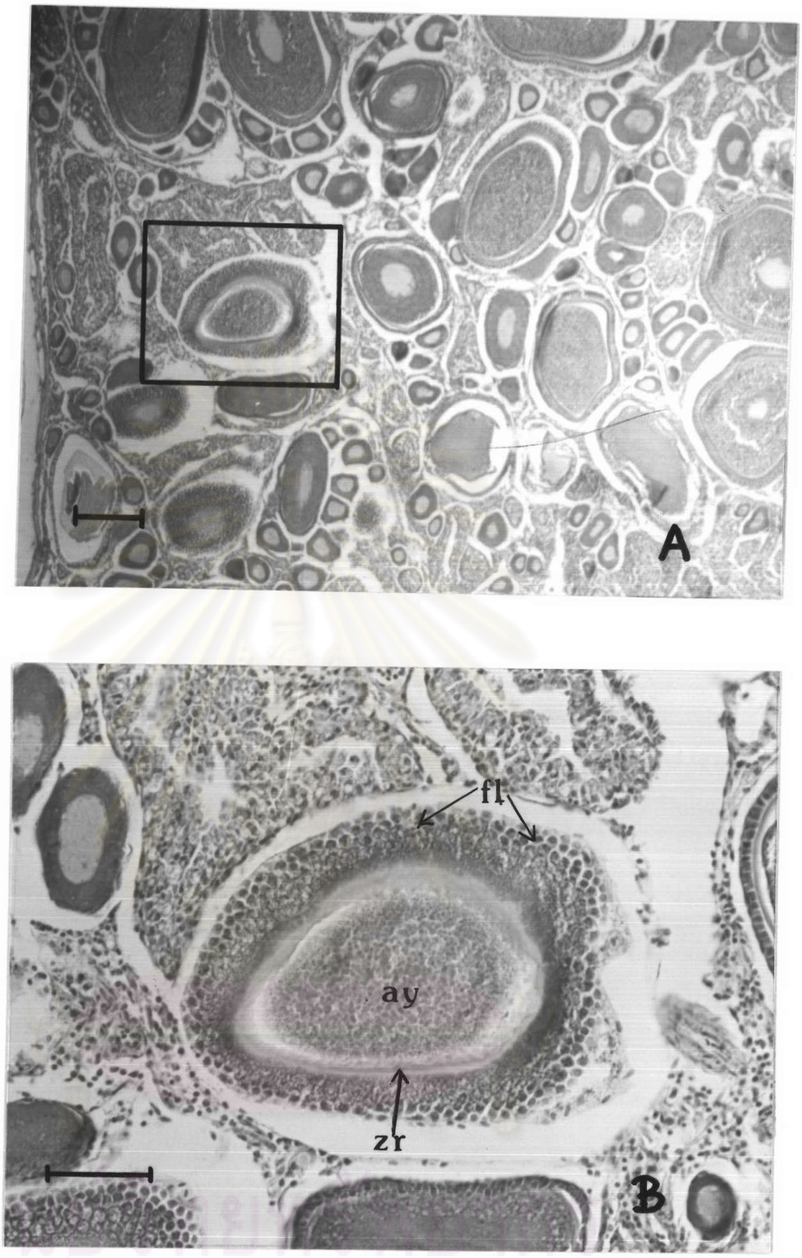

Figure 13. Atretic oocytes in the ovarian sections of *E. heteroloba*

ay: acidophilic yolk vesicles, zr: zonar radiata, fl: follicular cell

(Buffered Formalin; Haematoxylin and Eosin)

Bar scale: A = 100 μm, B = 50 μm

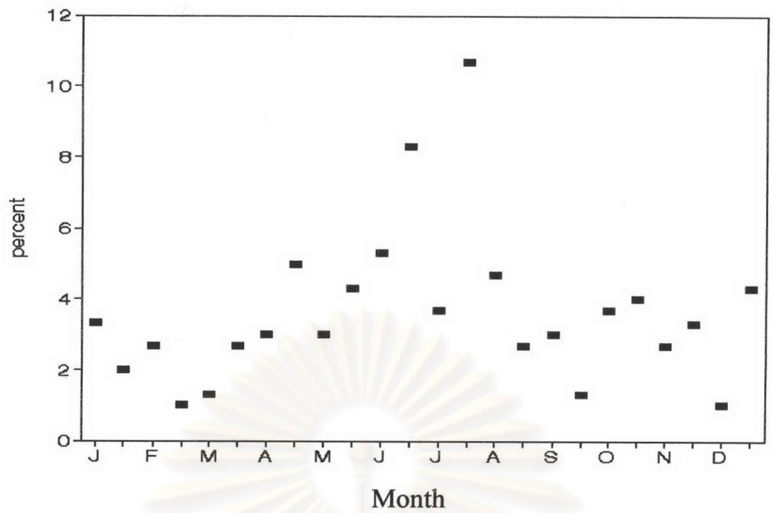

Figure 14. Percent of atretic oocytes in the ovaries of shorthead anchovy for each sampling period during 1995

4. <u>Cycle of Oocyte Development</u>

All stages except ripe oocytes were found in the ovaries of *E. heteroloba* in all sampling periods. Immature oocytes were the most numerous in all ovaries. They occupied 36-70 % of oocytes in each section. Developing oocytes scattered with the proportions of 12-30% of oocytes during the year. Mature oocytes comprised 20-37 % of oocytes at the beginning and the end of the year, and 15-20 % with some low peaks from late March to October reaching a minimum of 8 % in early June. Proportion of each oocyte stage was summarized in Figure 15. Microscopic appearance of ovarian sections in each spawning peak were shown in Figure 16.

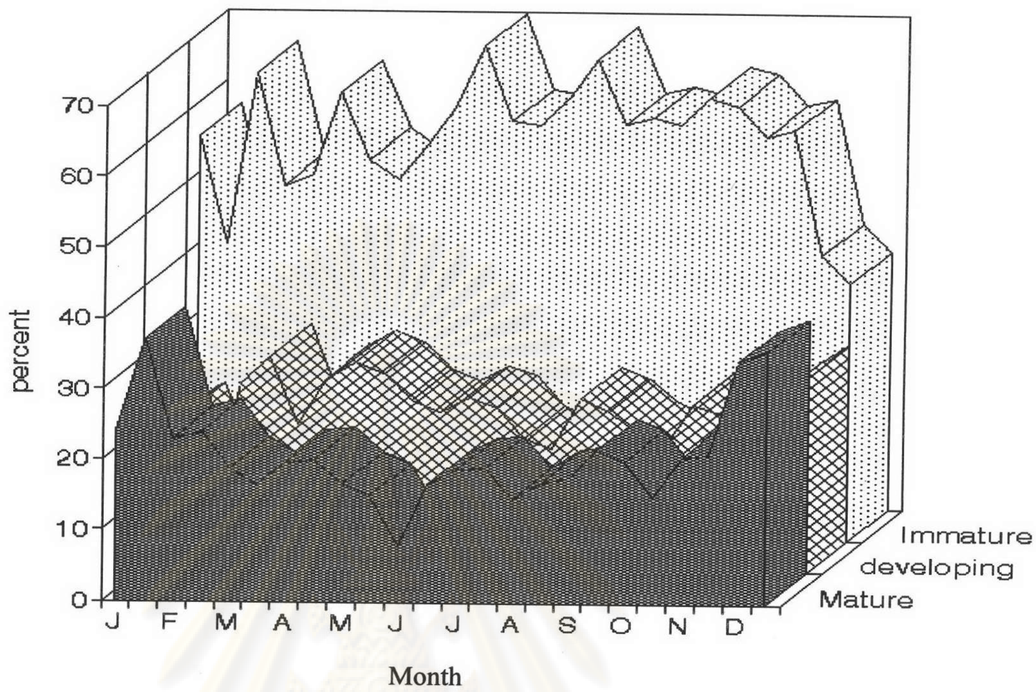

Figure 15. Variations of each oocyte stage in the ovaries of shorthead anchovy during 1995

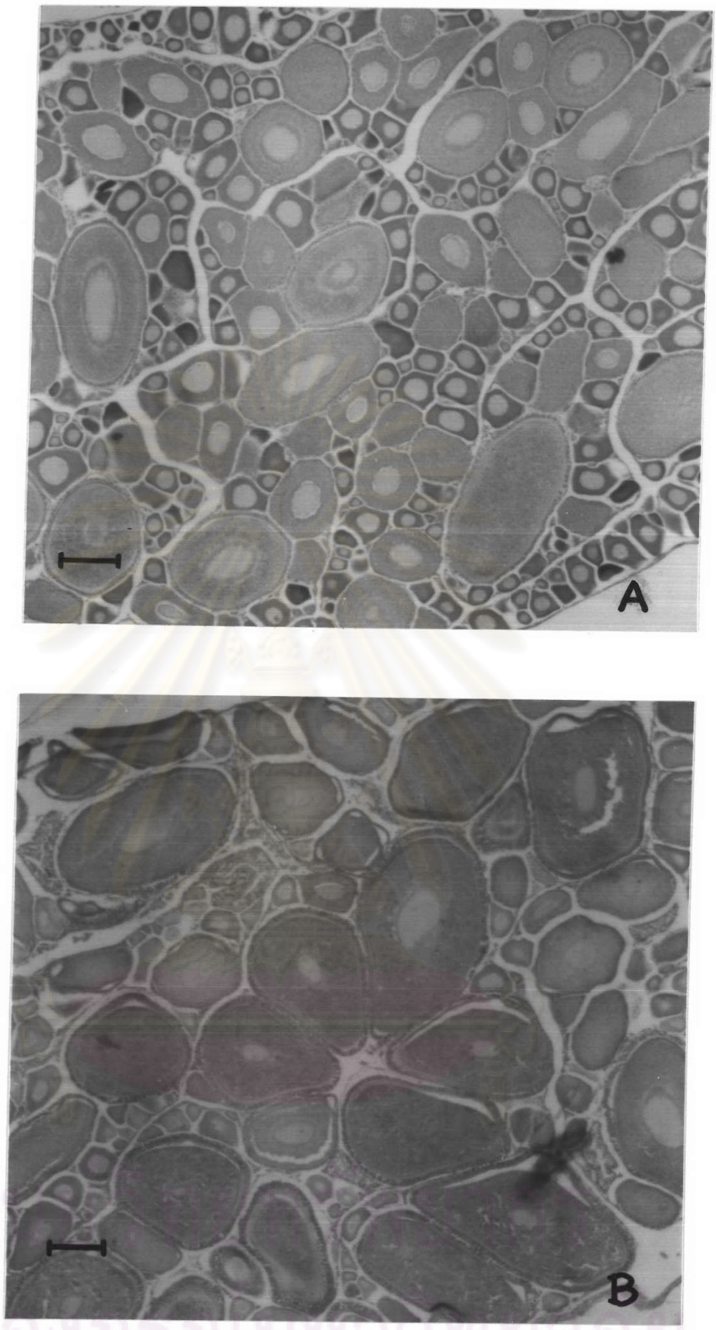

Figure 16. The ovarian sections of *E. heteroloba* in low spawning peak showing the dominance of immature and developing oocytes (A), and in high spawning peak showing the dominance of mature oocytes (B) (Buffered Formalin; Haematoxylin and Eosin)

Bar scale: = 100 μm

5. Oocyte Size-Frequency Distribution

Oocyte size-frequency distribution of *E. heteroloba* in each month (Figure 17) had no separated mode showing asynchronous developing of oocytes. This pattern indicated the continuous spawning in this species.

6. Oocyte Stage-Frequency Distribution

Oocytes at developing and mature stages were present in the ovaries of all sampling periods (Figure 18) showing the continuous recruitment of primary oocytes into the advanced stages. Ripe ovaries with hydrated oocytes were not found, and immature oocyte were excluded from the calculation as they were regarded as the reserved stock of oocytes commonly found in all ovaries of the fish.

7. Environmental Data

Data on rainfall and temperature in Rayong area obtained from Rayong Meteorological Station were compared to annual maturity of the fish, males and females combined (Figure 19). Percent of mature fish was inversely correlated with the amount of rainfall and atmospheric temperature. Season and rainfall were thought to affect spawning activity in this anchovy as more mature females occurred in dry season, the period of Northeast Monsoon, and low numbers occurred in the remaining period with some small peaks along with the fluctuation of rainfall.

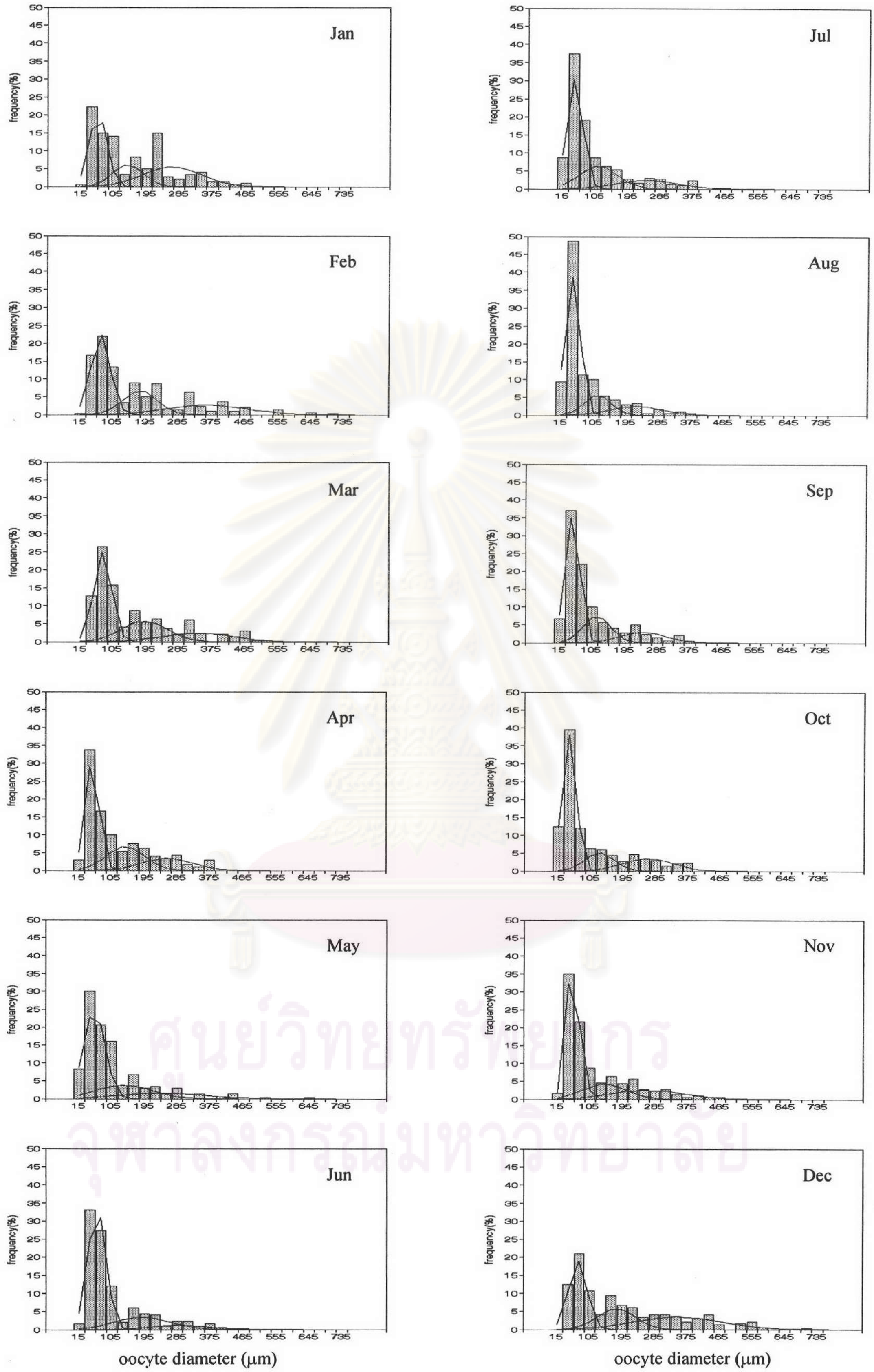

Figure 17. Oocyte size-frequency distributions in the ovaries of shorthead anchovy during 1995 showing total size frequency (bars) and expected stage frequency (lines): immature, developing, and mature stages

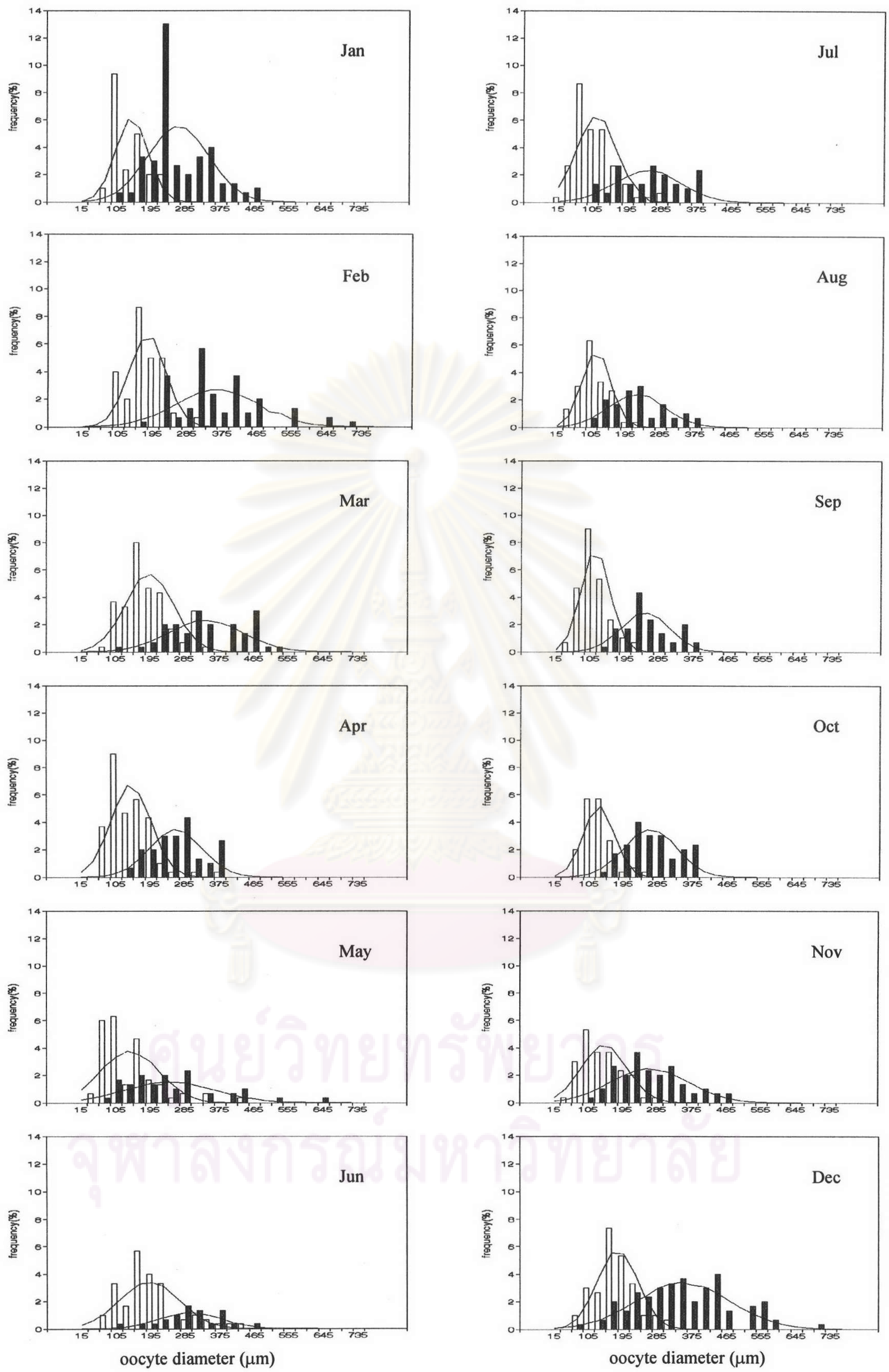

Figure18. Oocyte stage-frequency distributions in the ovaries of shorthead anchovy during 1995 showing frequency of developing stage (empty bars) and mature stage (filled bars)

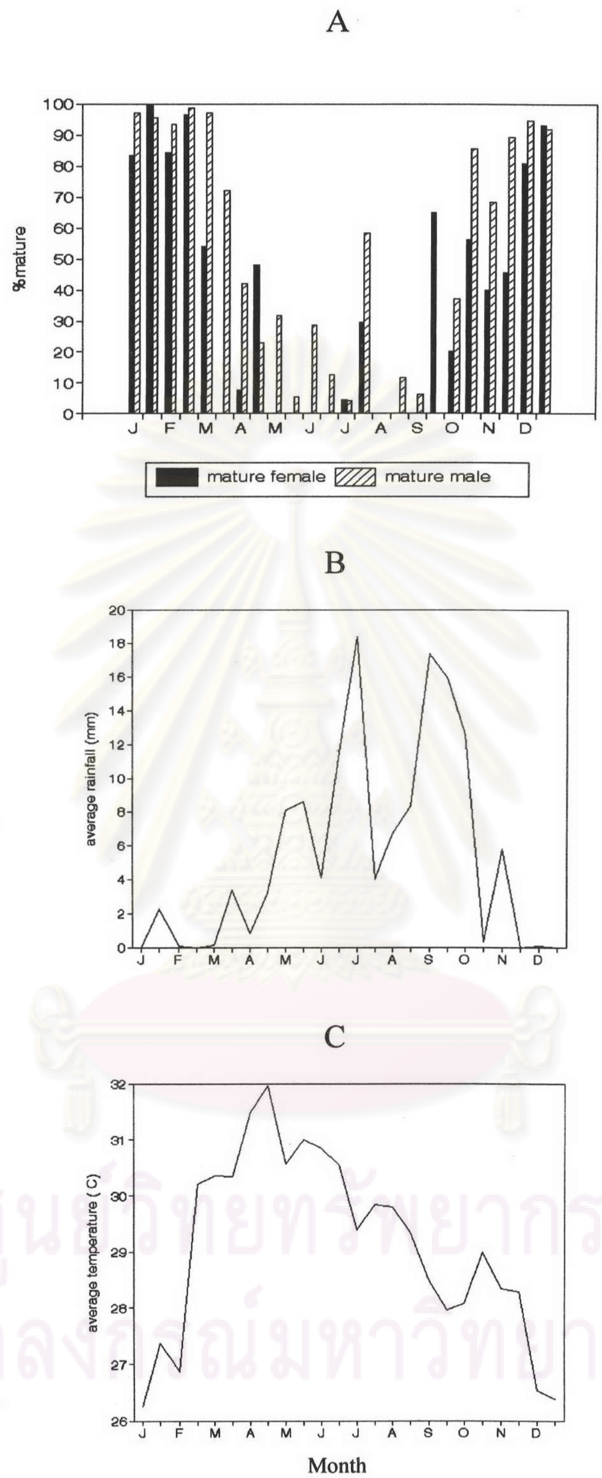

Figure 19. Variations of mature female and male shorthead anchovy (A), annual rainfall (B), and annual temperature (C) at Rayong in 1995