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DEVELOPMENT OF OOCYTES TO MATURITY AND SPAWNING

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Spawning in fishes is closely associated with the development of intra-ovarian eggs. Measurements of diameters of intra-ovarian eggs have been found to be an useful tool in studying the development of oocytes to maturity and spawning. An attempt to study the maturity by the measurements of ova was first made by Clark (1934) on the California sardine (Sardina caerulea). Her pioneering work was followed by those of Hickling and Rutenberg (1936) and De Jong (1939).

The method proposed by the above workers is essentially as follows: Ovaries are fixed in 5% formaldehyde and a small portion of the ovary is teased out on a slide in the same medium and measurements of all the ova in the field of the microscope are made, until about 500 ova are measured. In case the eggs appear asymmetrical due to preservation, the micrometer may be placed in horizontal position and the diameters are measured parallel to the graduations on the micrometer. Ovaries after fixing in Bouin's solution, may be cut in rotary microtome and the diameter of the oocytes in the sections could also be measured. Prabhu (1956) suggests that measurements of at least 1000 eggs from each ovary are necessary to mitigate the probable error in the representation of various groups of eggs in different stages of maturity and represented by various modes in the graphs. Normally the first batch of immature eggs are avoided in the measurements.

The intra-ovarian eggs very not only in their size but also in their inclusions in ovaries which are fully

ripe or in the penultimate stage of ripeness. There are several batches of oocytes which take their origin from the germ cells of the ovigerous lamellae and, as the spawning process continues every season, these batches pass on from one stage to the other. An examination of the ovary in the penultimate stage of development shows chiefly the following four types of ova: Fig. 3.3.1.

(1) Immature ova: ~~minute transparent ova~~ minute transparent ova possessing a nucleus and a protoplasmic layer.

(2) Maturing ova: small, opaque ova in which yolk formation has just commenced, but not completed.

(3) Mature ova: Opaque ova, fully yolked, but still contained within the follicle.

(4) Ripe ova: Large fully or partially transparent ova which have burst out from the follicles.

Ovarian maturity stages are determined based on the predominance of the above mentioned types of development.

Histologically, oocyte development could be broadly classified into a primary growth phase, a secondary growth phase and a final maturation to be followed by ovulation and spawning.

(1) Primary growth phase: The immature oocytes, known as the oogonia, are seen multiplicating by mitosis in the stroma of the ovigerous folds. The oogonia are transformed to the primary oocytes by arresting the chromosomes at the prophase of the first meiotic division; this process is known as oogenesis. In the oogonia, the nucleo-cytoplasmic ratio is high, but as the growth progresses, this ratio decreases. Highly spiralized lamp-brush chromosomes are usually seen in the nucleus of the primary oocyte. Nucleoli multiply and arrange along the periphery of the nucleus, called peri-nucleolar stage. The follicular

wall is thin and less prominent in the oocytes during primary growth phase (Fig. 3.3.2).

(2) Secondary growth phase: Appearance of different types of yolk bodies in sequence is the characteristic feature of the secondary growth phase. Normally carbohydrate-rich cortical alveoli make their appearance along the periphery of the oocyte. Oil droplets are present in most of the marine fish oocytes. The protein laden yolk appear in the form of yolk granules and these along with the lipid yolk fill the entire ooplasm during the advanced stages of secondary growth phase. Size of the oocyte increases considerably and follicular wall becomes prominent (Fig. 3.3.3).

(3) Final maturation: Primarily, oocyte maturation involves the resumption of meiosis. It is commonly regarded that the chromosomal activity proceeds to the metaphase of the second meiotic division. Resumption of meiosis is heralded by a peripheral migration of the germinal vesicle (nucleus) and by the dissolution of the germinal vesicle (Germinal Vesicle Breakdown or GVBD). GVBD is commonly used as an indicator of oocyte maturation. In some species, coalescence of yolk granules resulting in the oocyte becoming increasingly transparent. Concomitant with maturation in many teleosts, especially marine forms with pelagic eggs, oocyte enlargement is due to hydration. Hydration in some species may result in a 300-400% increase in oocyte volume.

The present contention is that oocyte maturation and ovulation are dependent on pituitary gonadotropin. Concomitant with maturation or after maturation is complete, oocytes are ovulated into the ovarian lumen.

Various authors have classified the different maturity stages into 4 to 7 stages, calling them as "Stage I","Stage II", etc., and taking into account the dominant size of the ova as well as the gross volume of the ovary in relation to the body cavity (Clark, 1934; Hickling and ~~Buteri~~Ruteriberg,

1936; Prabhu 1956, Nair, 1959; James and Badrudeen, 1981)

Based on ova diameter measurements, four different types of spawning periodicities have been recognized in teleostean fishes:

Type A: Spawning taking place only once a year during a definite, short period. In this case, the eggs which are destined to be spawned are withdrawn from the immature stock in a single group, sharply distinguishable at least in the later stages of maturation from the stock of small eggs from which it was derived (Fig. 3.3.5). The oocyte development in this case is said to be synchronous, as in Therapon jarbua, Macrones vittatus and Chirocentrus dorab.

Type B: Spawning taking place only once a year, but with a longer duration. In species exhibiting this type of spawning, the range in size of the mature ova, irrespective of the number of modes representing them, have been found to be nearly half of the total range in size of the entire intra-ovarian eggs in the whole ovary (Fig. 3.3.4), as in Pelates quadrilieatus and Cypsilurus oligolepis.

Type C: Spawning twice in an year. In the ovaries of fishes exibiting this type of spawning, in addition to the batch of eggs in ripe condition, another batch of eggs in which yolk formation has already commenced could be seen (Fig. 3.3.4), as in Psammoperca waigensis, Therapon puta and Selaroides leptolepis.

In the B and C types of spawning, the oocyte development is described as group synchronous.

Type D: Spawning throughout the year, but intermittently. Withdrawal of eggs from the immature stock is a continuous process; and there will be no sharp separation between the general egg stock and the maturing eggs (Fig.3.3.5). The pattern of oocyte development in this case is said to

be 'asynchronous', as observed in Stolephorus indicus.

It may be mentioned here that a protracted spawning season cannot always be equated with multiple spawns for each female. Protracted spawning season may simply reflect lack of population synchrony in terms of gonadal development. The term multiple spawner is generally applied to a species in which a female spawns more than once in a spawning season. The term fractional spawner has been used to refer to a species which spawns a part of the ovulated clutch or which mature ovulate, and spawn a part of a post-vitellogenic clutch at intervals over a relatively short period.

Clark (1934) points out that if only one batch of eggs is spawned, the ratio between the number of eggs in the maturing group and the number of eggs in the mature group should remain constant and on the other hand, if more than one batch is spawned, the ratio gradually decreases. Based on this principle she proves that individual California sardine spawns an average of three batches. For inferring multiple spawning, she provides four lines of evidence, viz., multiplicity of modes in the ova diameter frequency curves, a high degree of correlation between the growth of successive groups of eggs, occasional presence in the ovary of a few ripe, unspawned eggs and decrease in the ratio of the number of eggs in the maturing and mature groups as the breeding season advances.

Fertilisation

When the egg becomes ripe, a small opening known as the micropyle appears on it, through which the sperm enters at the time of fertilisation. It is assumed that soon after fertilisation, water enters through the pores of the egg membrane, lifting it from the yolk to form the perivitelline space. It is not known whether before fertilisation the micropylar canal is closed by a substance requiring an enzyme from the sperm to break it down.

When the egg is laid free in the water, the outer covering (chorion) at once becomes hardened. The hardened chorion becomes thinner as development advances and the egg increases in size. This process called 'water hardening' is advantageous in that it offers protection to the eggs from predators. It is assumed that a part of the substance of the egg membranes is withdrawn and absorbed by the enbryo and this seems to occur to a greater extent in the demersal eggs, where the embryo is more advanced on hatching, than in the pelagic eggs.

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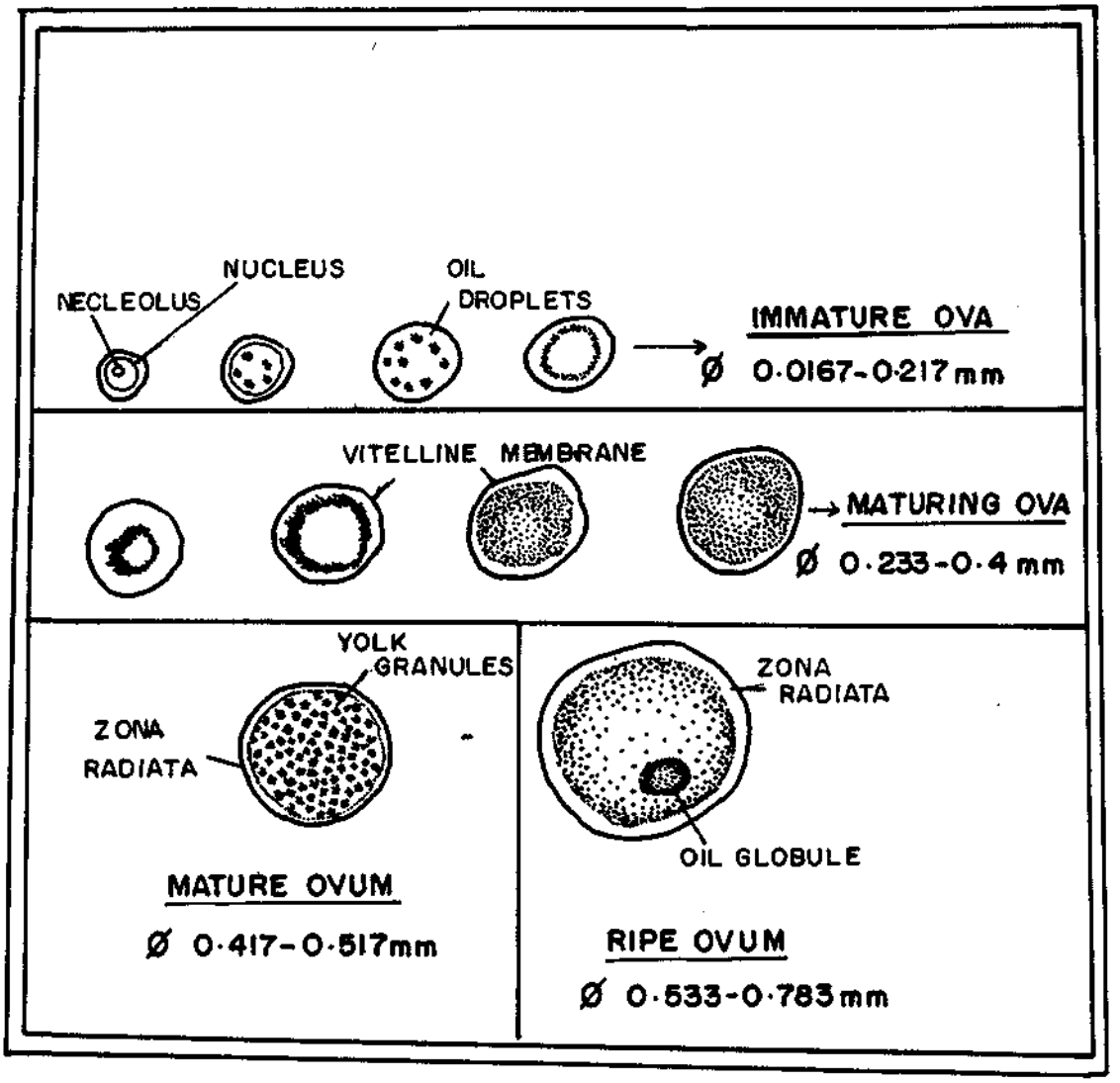
Fig.3.3.1


NUCLEUS
NECLEOLUS
OIL
DROPLETS
IMMATURE OVA
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VITELLINE MEMBRANE
MATURING OVA
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YOLK
GRANULES
ZONA
RADIATA
MATURE OVUM
Ø 0·417-0·517mm
ZONA
RADIATA
OIL GLOBULE
RIPE OVUM
Ø 0·533-0·783mm

PRIMARY GROWTH PHASE

Fig.3.3.2

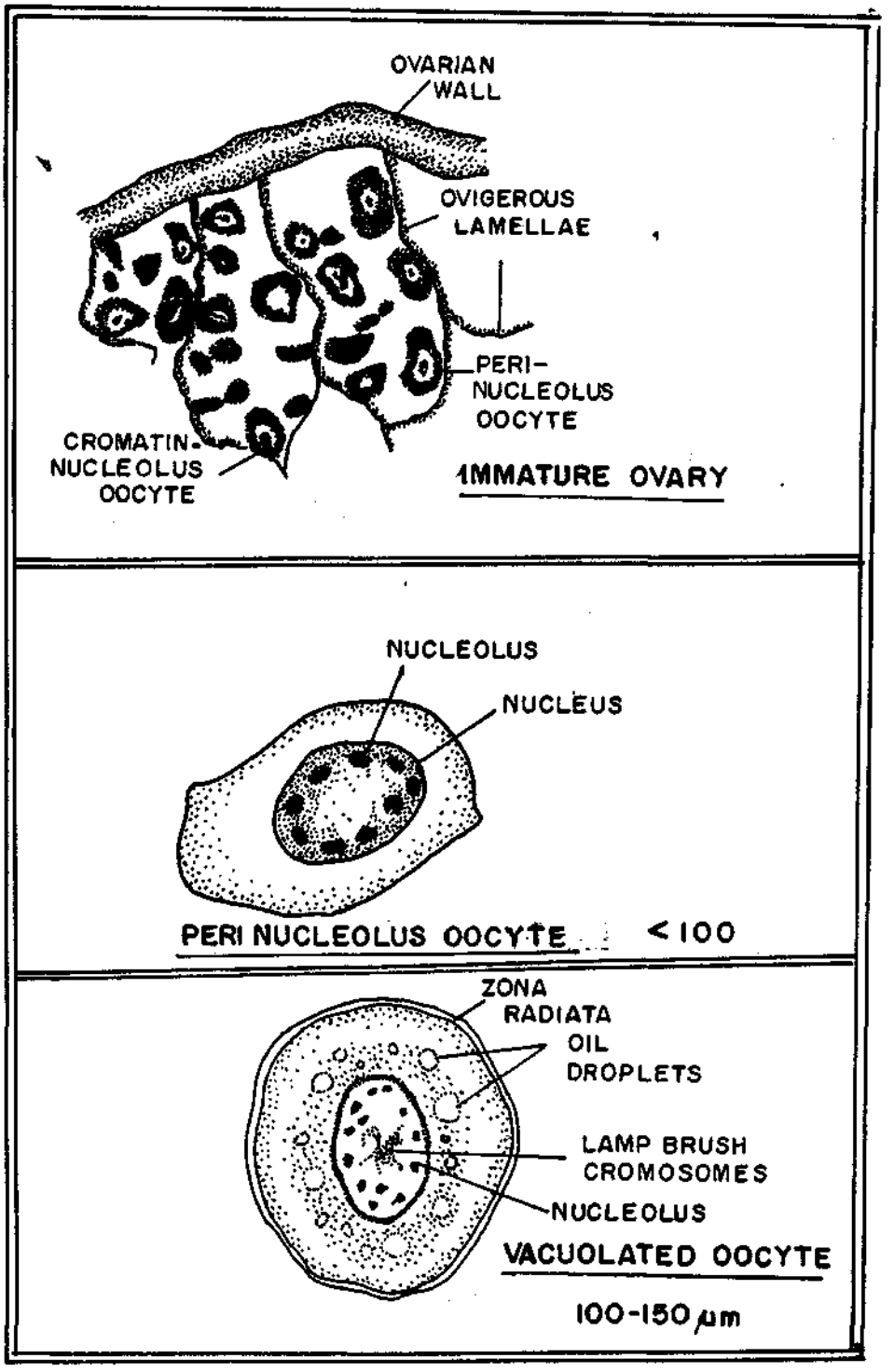


Fig. 3.3.3

SECONDARY GROWTH PHASE AND FINAL MATURATION

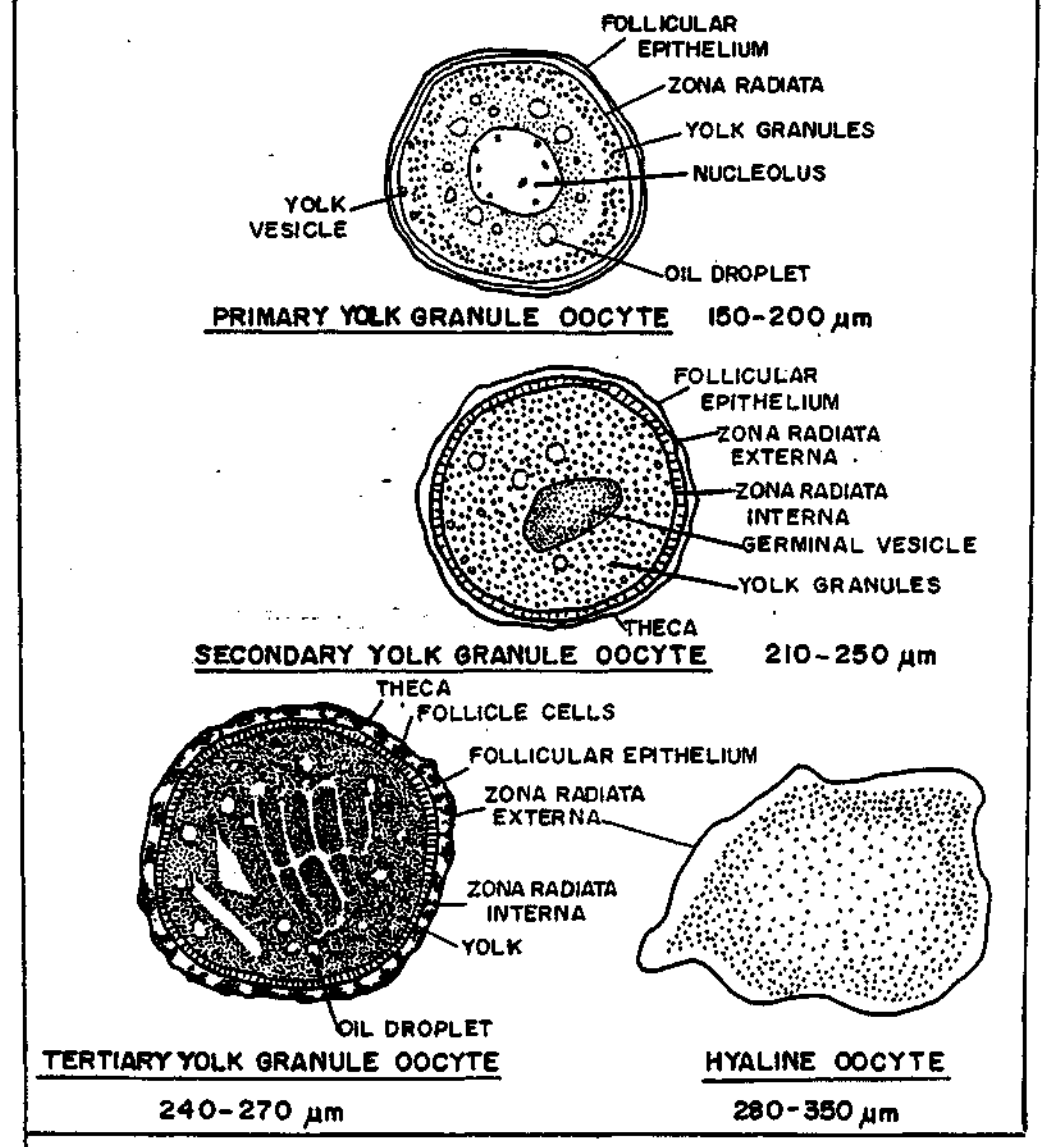
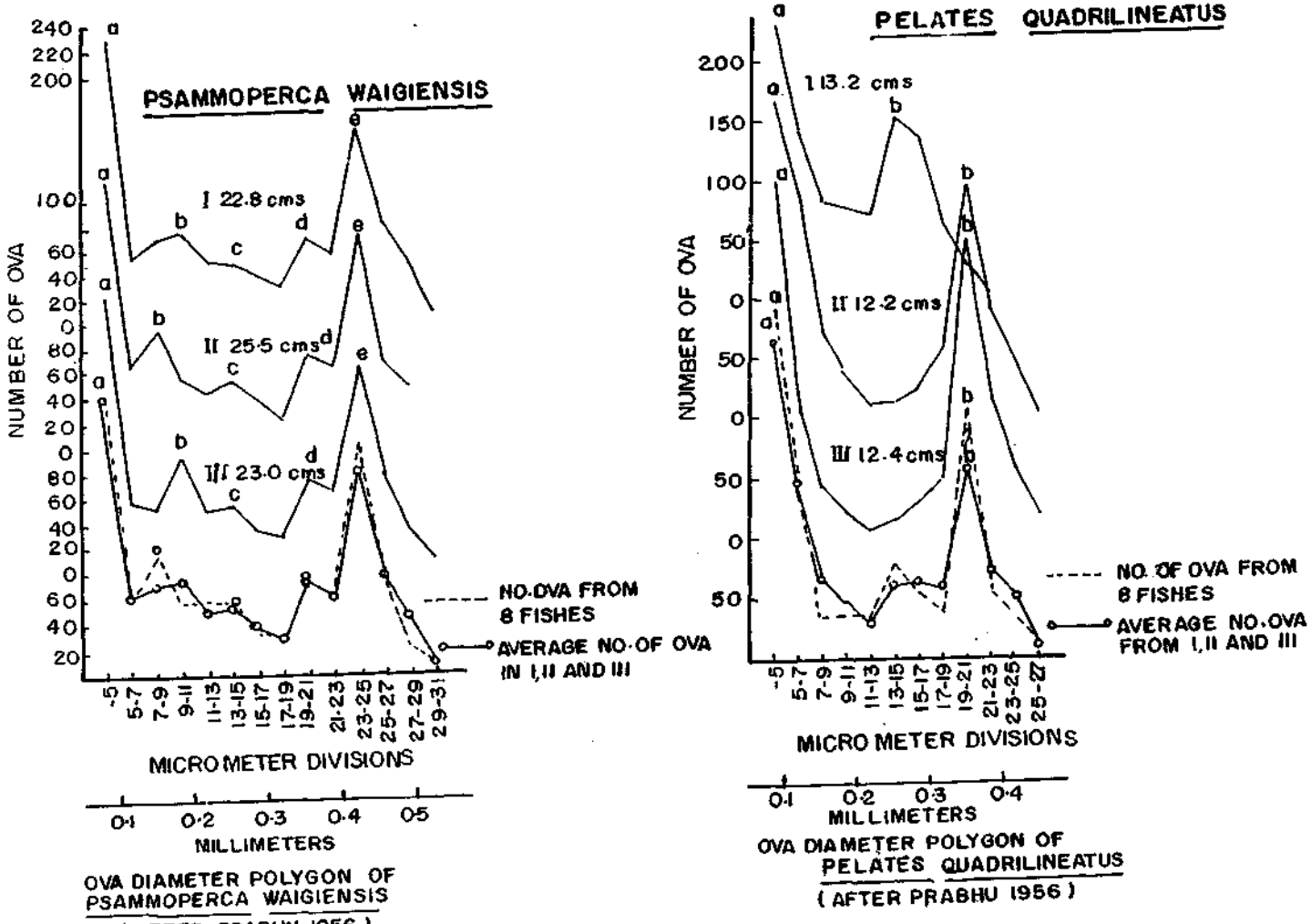


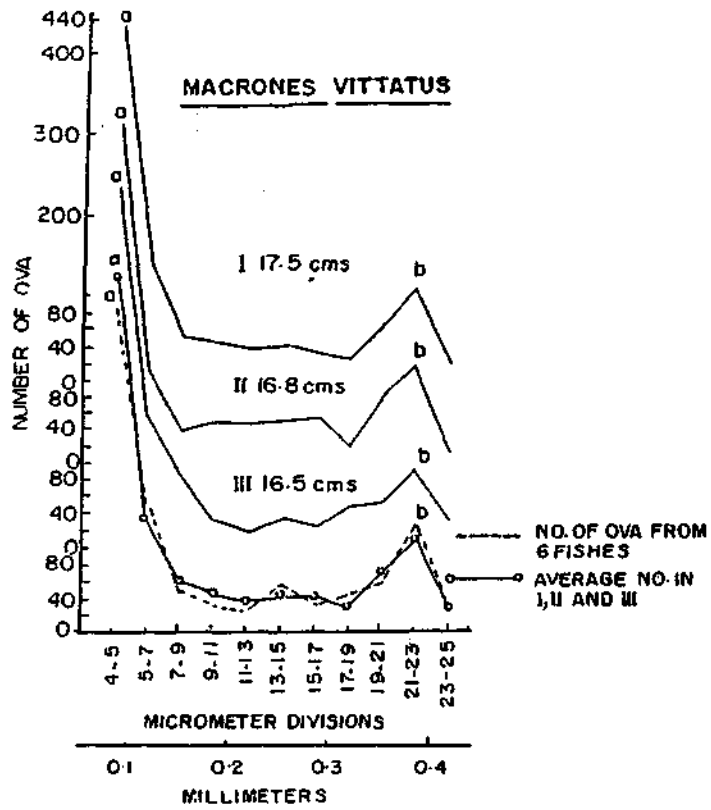
Fig.3.3.4



OVA DIAMETER POLYGON OF PSAMMOPERCA WAIGIENSIS
(AFTER PRABHU 1956)

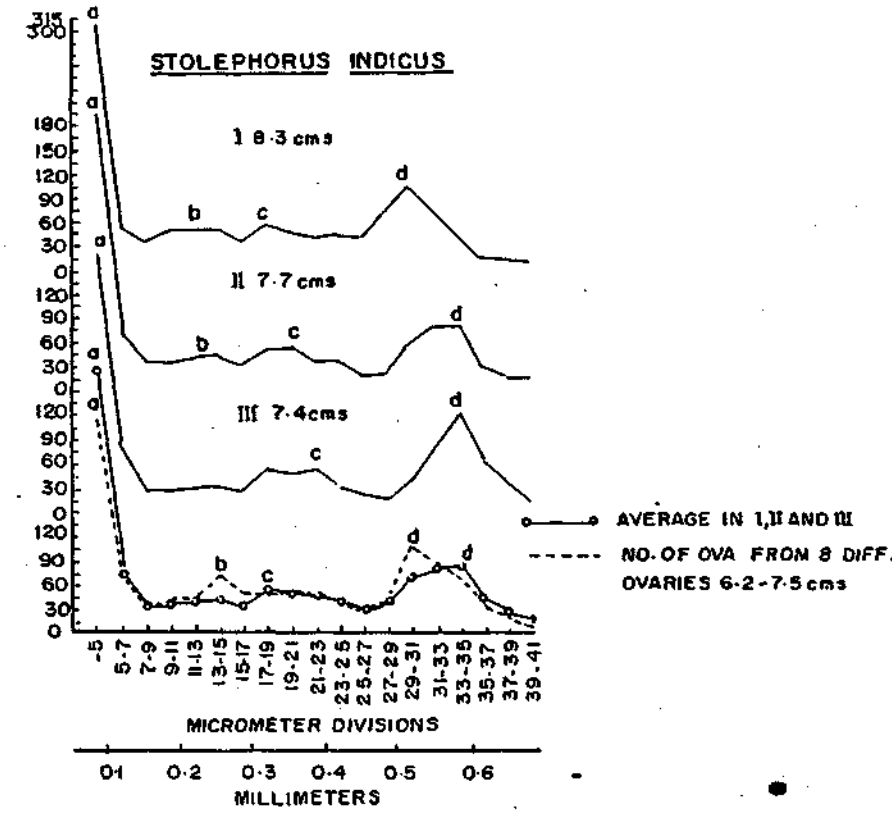
OVA DIAMETER POLYGON OF PELATES QUADRILINEATUS
(AFTER PRABHU 1956)

Fig.3.3.5



OVA DIAMETER POLYGON OF MACRONES VITTATUS

(AFTER PRABHU 1956)



OVA DIAMETER POLYGON OF STOLEPHORUS INDICUS

(AFTER PRABHU 1956)