Fish Reproduction and Development

Coevolved traits for producing another generation that will produce another generation…and another…and another…

Reproduction

Reproduction is the biological process by which new "offspring" or individual organisms are produced from their "parents". Reproduction is a fundamental feature of all known life; each individual organism exists as the result of reproduction

The known methods of reproduction are broadly grouped into two main types:

a) Sexual (Bisexual, Hermaphrodite)

b) Asexual (Parthenogenetic)

Type of Reproduction

1) Bisexual or heterosexual- most of the fish (Gonochoristic (single sex, fixed at maturity)

2) Hermaphrodite- ***Perca, Stezostedon, Micropterus***

- Simultaneous hermaphrodites function as male and female at same time (23 families; ex. Anguilliformes, eels; Atheriniformes, killifish)

- Sequential hermaphrodites start life as one sex, change sex after maturity
  Protandrous: male first, female later
  Protogynous: female first, male later (most common, Wrasses)

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3) Parthenogenetic (asexual)- (Amazon molly *Poecilia formosa)*

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In parthenogenetic reproduction, unfertilized eggs develop into embryos.

Gynogenetic – sperm needed for egg development, but mating without fertilization, result is daughters are genetic clones of mothers
Hybridogenetic - egg development with fertilization by males of *other* species, but male genes discarded at next generation

Reproduction Strategies

Based on reproductive strategies adopted by different fishes, they are categorised into three groups:

1. **Non guarders**
2. **Guarders**
3. **Bearers**

Non Guarders (they do not guard their egg and larvae) are of two types

i) **Open substrate spawners** (lay eggs in open places)
ii) **Brood Hiders** (lay eggs in hidden places)

Guarders (Male or Female or both the parents guard the eggs and larvae after laying)

i) **Substrate choosers**
ii) **Nest spawners**

Bearers

i) **External Bearers**
ii) **Internal Bearers**

Mating System

- **Promiscuous** - both sexes with multiple partners - most common
- **Polygamy**-Either male or female will have multiple partners in a given breeding season
- **Polygynous** - males with multiple mates (cichlids)
- **Polyandry** - females with multiple mates – few (Anglerfish, males “parasitize” females
- **Monogamy** - mating pair remains together over time, long gestation of young (some cichlids, seahorses, pipefish)

Method of fertilization

- most fishes use **external fertilization**
  - less time and energy in courtship, pair bonding
  - increases number of potential mates
  - greater fecundity

- **internal fertilization** in few groups:
  - sharks, rays, skates, ratfishes (Chondrichthyes)
  - guppies, mollies, etc. - Poeciliidae, Goodeidae
  - surfperches - Embiotocidae

Method of fertilization *cont*……

– internal fertilization requires
  - **lengthy courtship, preparation for mating**
  - **intromittent organ**
    - **claspers (pelvic fins) in Chondrichthyes**
    - **modified anal fin in poeciliids, goodeids**
    - **modified genital papilla in embiotocids**
  - **male structure for storing sperm (seminal vesicle)**

– buccal fertilization

Sperm swallowing (eg. Callichthyid catfish (*Corydoras*)

Reproduction Methods

1) Oviparous- egg layerer

Producing eggs that develop outside the maternal body (**many bony fishes)**

2) Live bearer

a) Viviparous

fertilization is internal and during gestation, there is maternal-embryonic transfer of nutrients. **Anablepidae, Jenynsiidae, Goodeidae, Poeciliidae**

b) Ovoviviparous

Fertilization is internal, but during gestation there is no maternal-embryonic exchange of nutrients and developing embryo sustain on yolk reserve of oocyte. **Scorpaenidae, Cottidae, Hexagrammidae**

In some ovoviviparous fishes the embryo develops in the egg while the egg is still within its follicular covering within the ovary, and ovulation (or release of the egg) and birth occur at the same time. In other ovoviviparous forms the eggs are released from the protective follicles into the cavity of the hollow ovary, where development continues. In some viviparous fishes the walls of the egg follicle are in intimate contact with the embryo, supplying it with nourishment. In the viviparous sharks, a part of the oviduct, or egg channel, is developed into a uterus, where the modified yolk sacs of the young are closely joined to pockets within the uterus.

Sexual Dimorphism

Most of the fishes exhibit sexual dimorphism or secondary sexual characters by which sex can be distinguished from each other. In few fishes secondary sexual characters are discernible throughout the life span and show some structural differences like

1. Show morphological peculiarities which facilitate fertilization of ova , as copulatory organ in male.
2. Structural peculiarities that are not related to fertilization but are meant for courtship and fighting with other males.

While in some fishes secondary sexual characters are discernible during breeding season and external morphological differences pertain to the following features

1. Size of fish
2. Length/shape/texture of fins
3. Coloration
4. Genital papilla
5. Ovipositor
6. Shape of mouth

Function of secondary sexual characters

a) Recognition of opposite sex, b) helping in the act of copulation such as sexual embrace, c) transfer of spermatozoa from male to female, d) facilitating parental care

Reproductive morphology

- **Cartilaginous fishes:**
  - male: testes -> Leydig's gland -> seminal vesicle -> cloaca -> claspers
  - female: ovary -> ostium tubae -> oviduct -> shell gland -> [uterus] -> cloaca
- **Most Bony Fishes:**
  - male: testes -> vas deferens -> urogenital pore
  - female: ovary -> oviduct -> urogenital pore

Reproductive organ

The fishes have one pair of bilateral gonad. Generally symmetrical.

Suspended from dorsal portion of body cavity by mesenteries in close association of kidneys. Mesentery is richly supported with blood vessels and nerve fibres.

In male mesentery is called mesorchium

In female mesentery is called mesovarium

Male Reproductive Organ

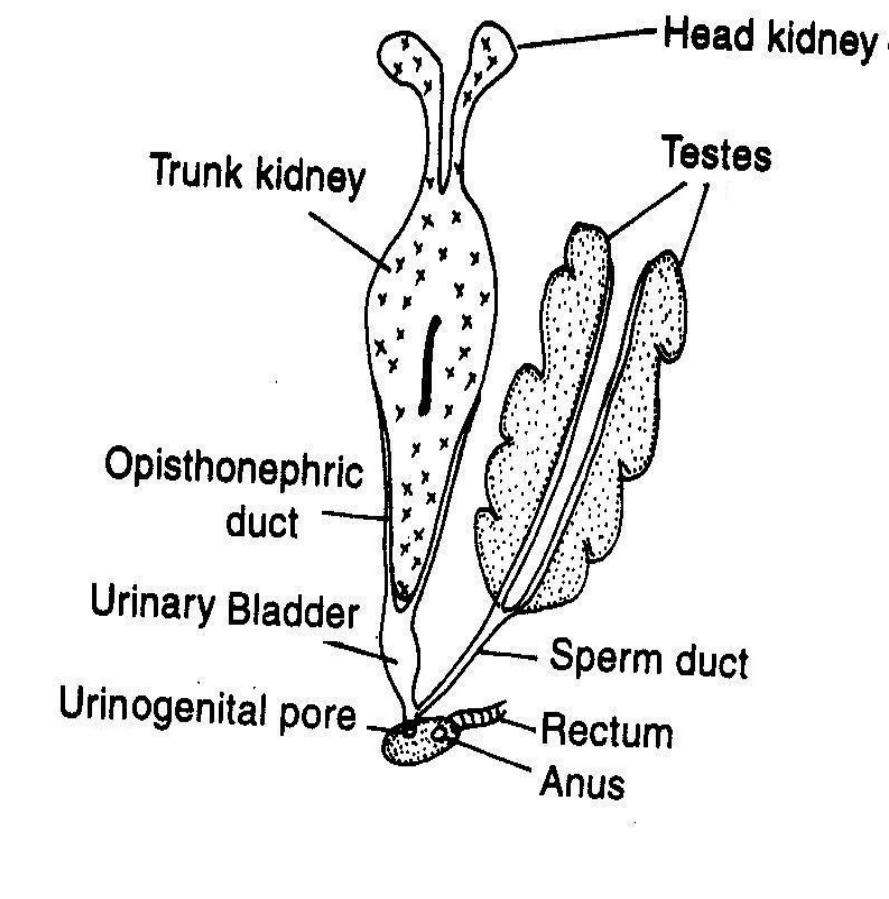
Male reproductive organ Composed of

i) Testis

It is paired, elongated, flattened structure present on either side of ventral to kidney in the abdominal cavity. Attached to the body wall by means mesorchium

ii) Sperm duct or vas deferens

iii) Genital pore or cloaca



Testis

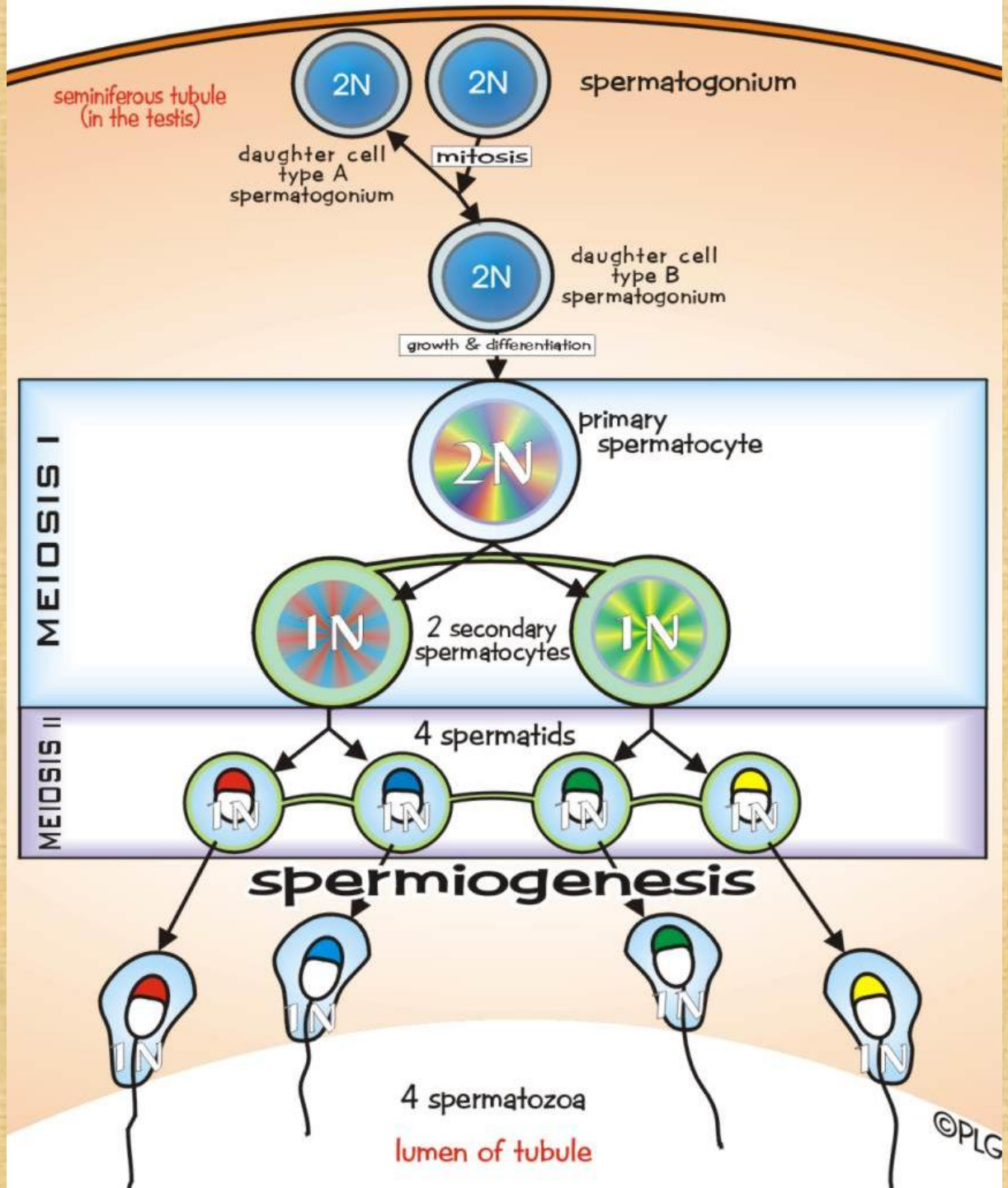
In most cases, testes are a pair of elongated structures composed of numerous seminiferous tubules embedded in the stroma.

The testis consists of thin-walled tubules or lobules that contain germ cells - the spermatogonia - which are endodermal in origin. Germ cells divide in clusters enclosed by a cyst.

Primary spermatogonia - which are present throughout the year, divide mitotically to give rise to secondary spermatogonia which get transformed into primary spermatocytes.

They divide by meiosis and give rise to spermatids from which spermatozoa are formed.

The seminiferous tubules are packed with spermatozoa in the pre-spawning and spawning periods,



Development of sperm or spermatozoa from primary germ cell or spermatogonium is called spermatogenesis

Spermatids undergo metamorphosis to produce sperm and this process is called spermiogenesis

Structure of sperm

a) Head piece

b) Middle piece

c) Tail

In fish sperm, acrosomes are absent

Reproductive cycle (Testicular)

Most of the fishes breed in one season every year (**seasonal spawners**) and some breed through out the year (**year round spanwers**). Seasonal breeders exhibit rhythmic changes in the structure and physiology of ovary and testes in different seasons. These changes in testis are demarcated into five phases

1. Resting phase: Testis remain in immature state, Seminiferous tubules are solid being filled with spermatogonial cells

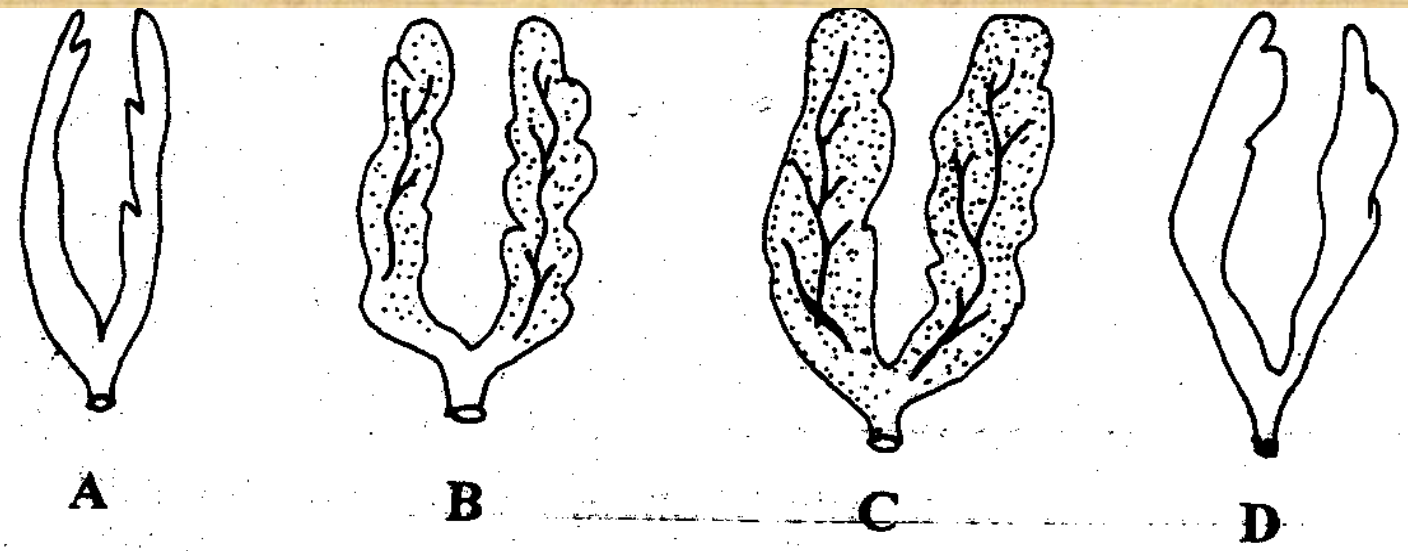
2. Preparatory phase: During this phase cell proliferation and 1st and 2nd meiotic division occur as a result primary spermatocytes, secondary spematocytes and spermatids are produced **(spermatogenesis)**.

3. Mature Phase: during this spermatids undergo further development and metamorphosis and develop into mature spermatozoa or sperm. The preocces of development of spermatozoa from spermatids is called **spermiogenesis**.

4. Spermiation Phase: During courtship and mating process, male eject milt (spermatozoa in seminal fluid) out of its body through genital aperture to fertilized the eggs released by female. The seminal fluid is mostly secreted by the cells lining the vas deferens (sperm duct) and provide nourishment to the mature sperm. Both spermatogenesis and seminal fluid secretion are under control of gonadotropin of pituitary gland and male hormone testosterone.

5. Post spermiation phase: during this phase testis is characterized by the presence of evacuated seminiferous tubules.

Maturity stages of testes



Showing seasonal changes in the testes; **A** Immature (Resting), **B** Maturing testis, **C** Mature testis, **D** Spent testis.

Female Reproductive Organ

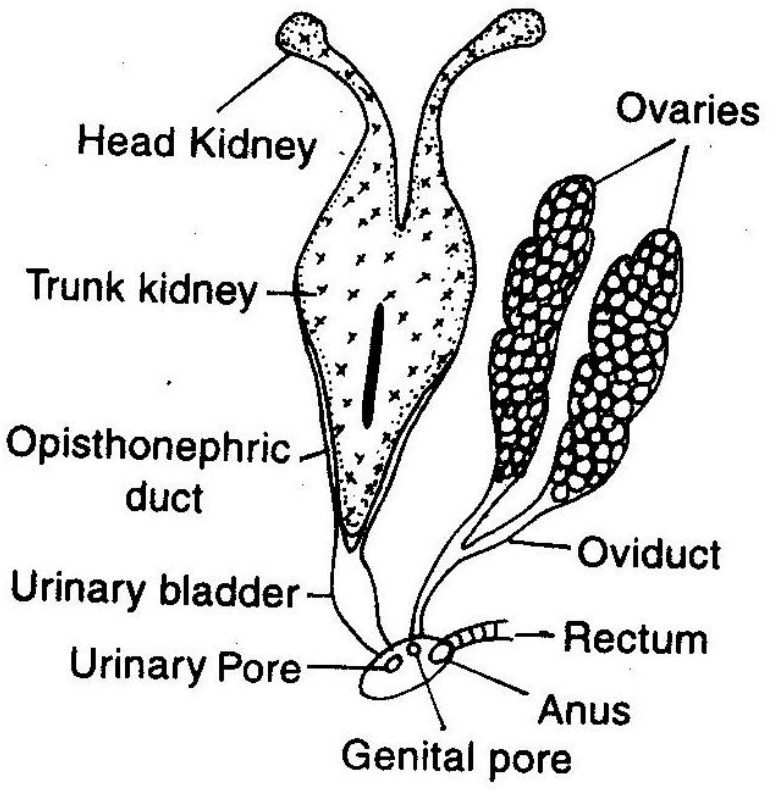
Female reproductive organ Composed of

i) Ovaries

It is paired, elongated sac like structure present on either side of ventral to kidney in the abdominal cavity. Attached to the body wall by means mesovarium

ii) Oviduct

iii) Genital pore or cloaca



Types of ovary:

1.Cystoarian: mature oocytes released in to ovoceal that is continued as oviduct to open into exterior (*Lepidosteus* and Teleost fishes)

2. Gymnoarian: mature oocytes released into body or coelomic cavity from where they passout through genital aperature (Chondrichthyes, Dipnoi, Chondrostei and *Amia*)

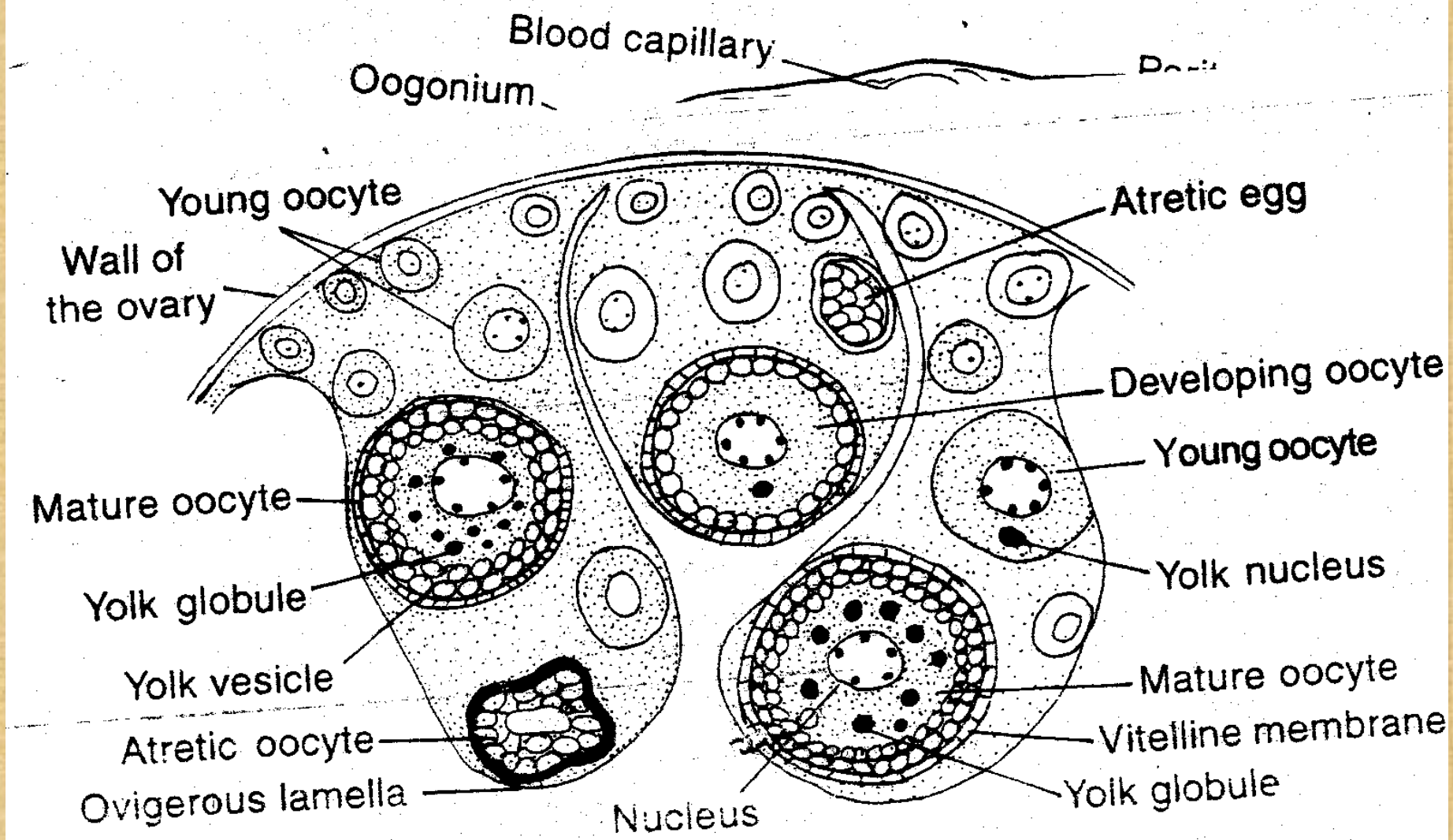
Ovary

The ovary in most teleost fishes is a hollow sac-like organ into which extend numerous ovigerous folds (lamellae) lined by germinal epithelium.

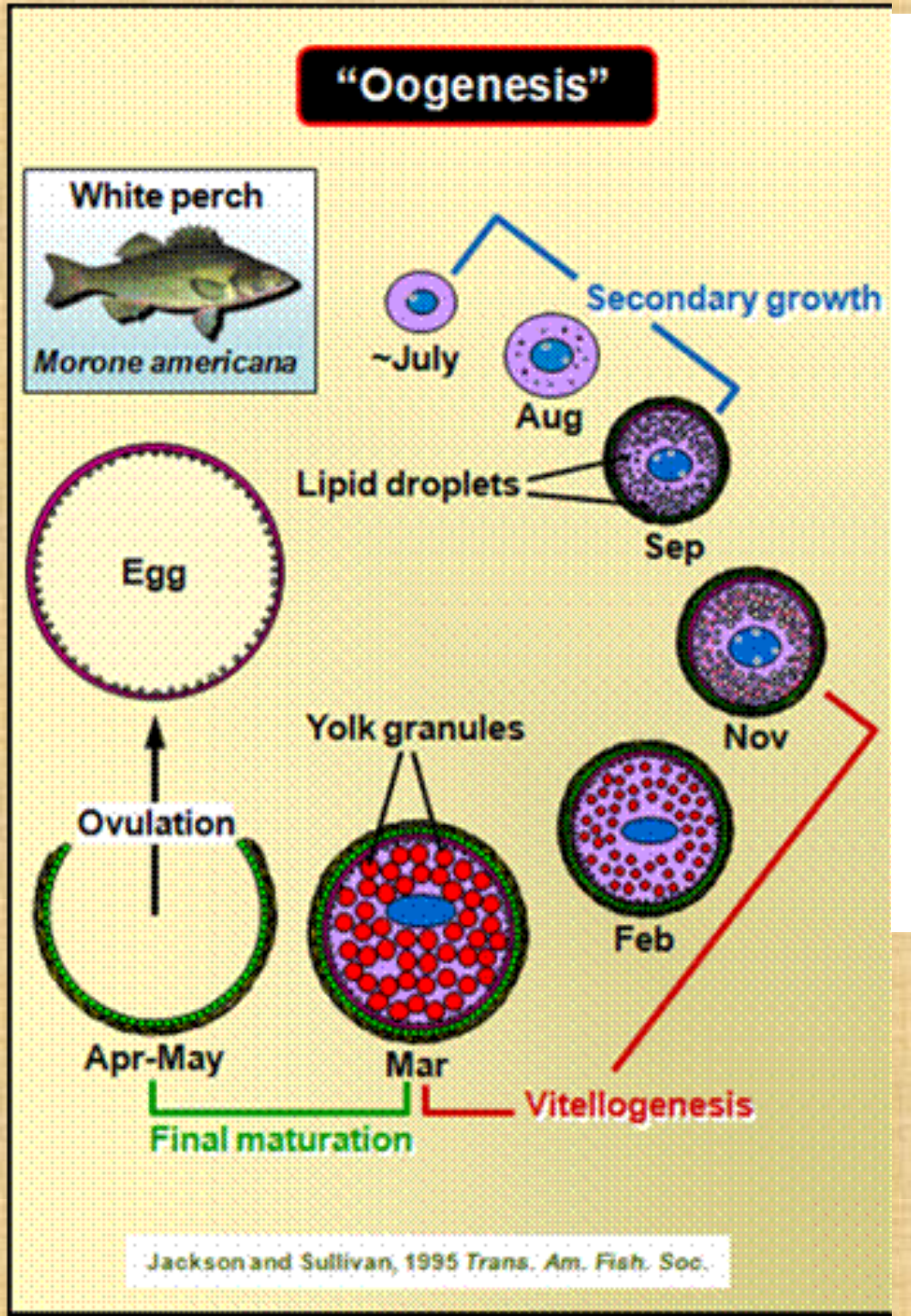
The germ cells, oogonia (originate from germinal epithelium) multiply mitotically and get transformed into non-yolky primary oocytes

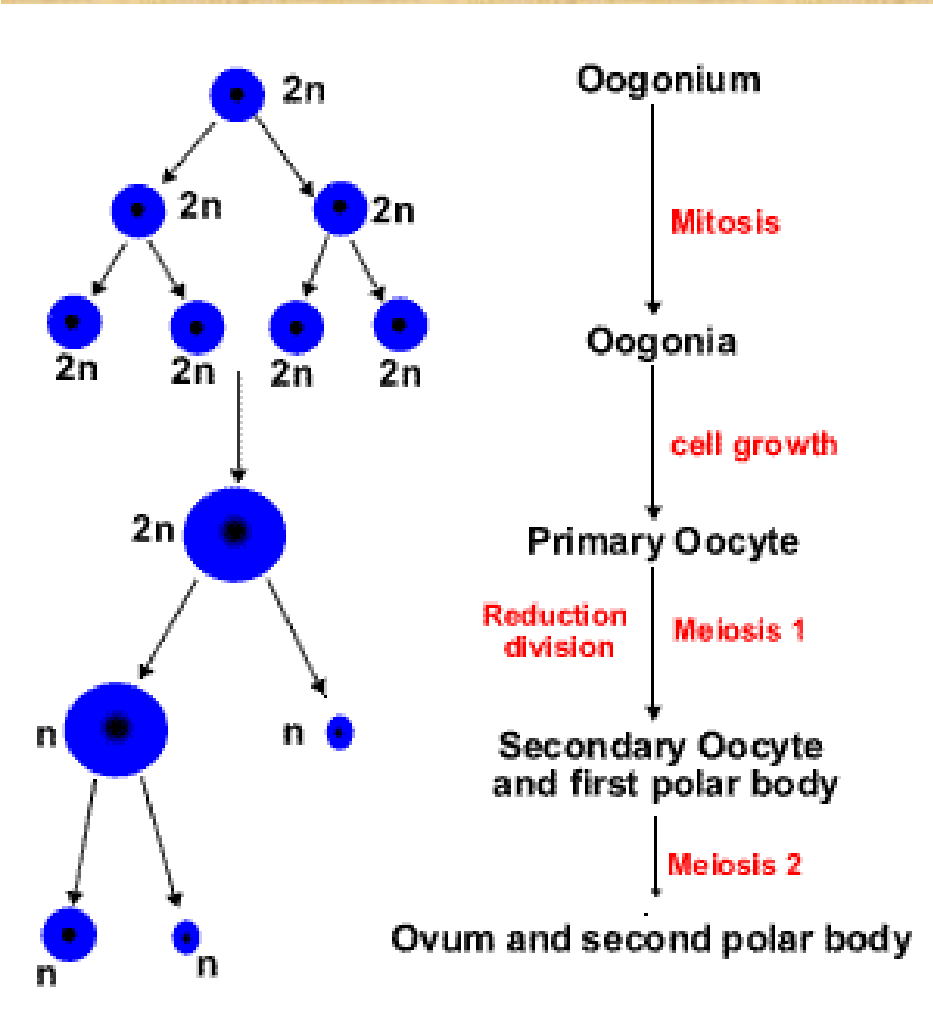
Primary oocytes, covered generally by two layers of follicle cells, an outer thecal layer and an inner granulosa layer, undergo vitellogenesis when yolk is deposited in the ooplasm.

Analysis of yolky eggs indicates that the egg-yolk is composed of lipovitellin and phosvitin, the former is rich in lipid and poor in phosphorus and the latter is rich in phosphorus and poor in lipid



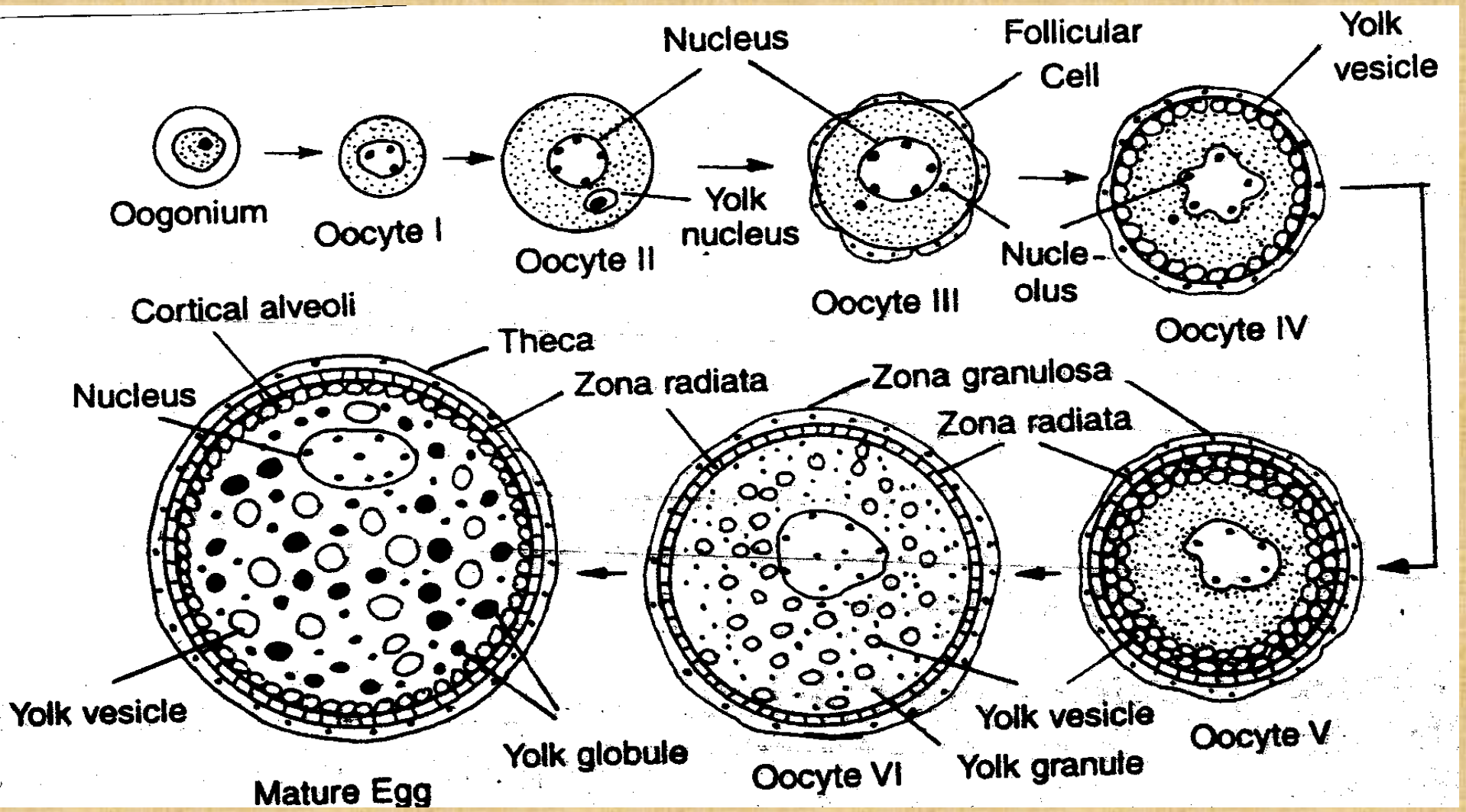
T.S. Mature ovary.





Process of development of mature egg from primordial germ cells called oogenesis

Stages in the maturation of oocytes



Reproductive Cycle (Ovarian)

Parallel to testicular cycle, the primary reproductive organ of female i.e. ovary also undergoes rhythmic change which is divided into five phases as follows:

1. Resting Phase: the ovary is in immature state containing nests of oogonial cells stage 1 oocytes at different phases of growth and few stage II oocytes. **During this stage the first meiotic division of nucleus is initiated** and the same is arrested at pachytene stage.

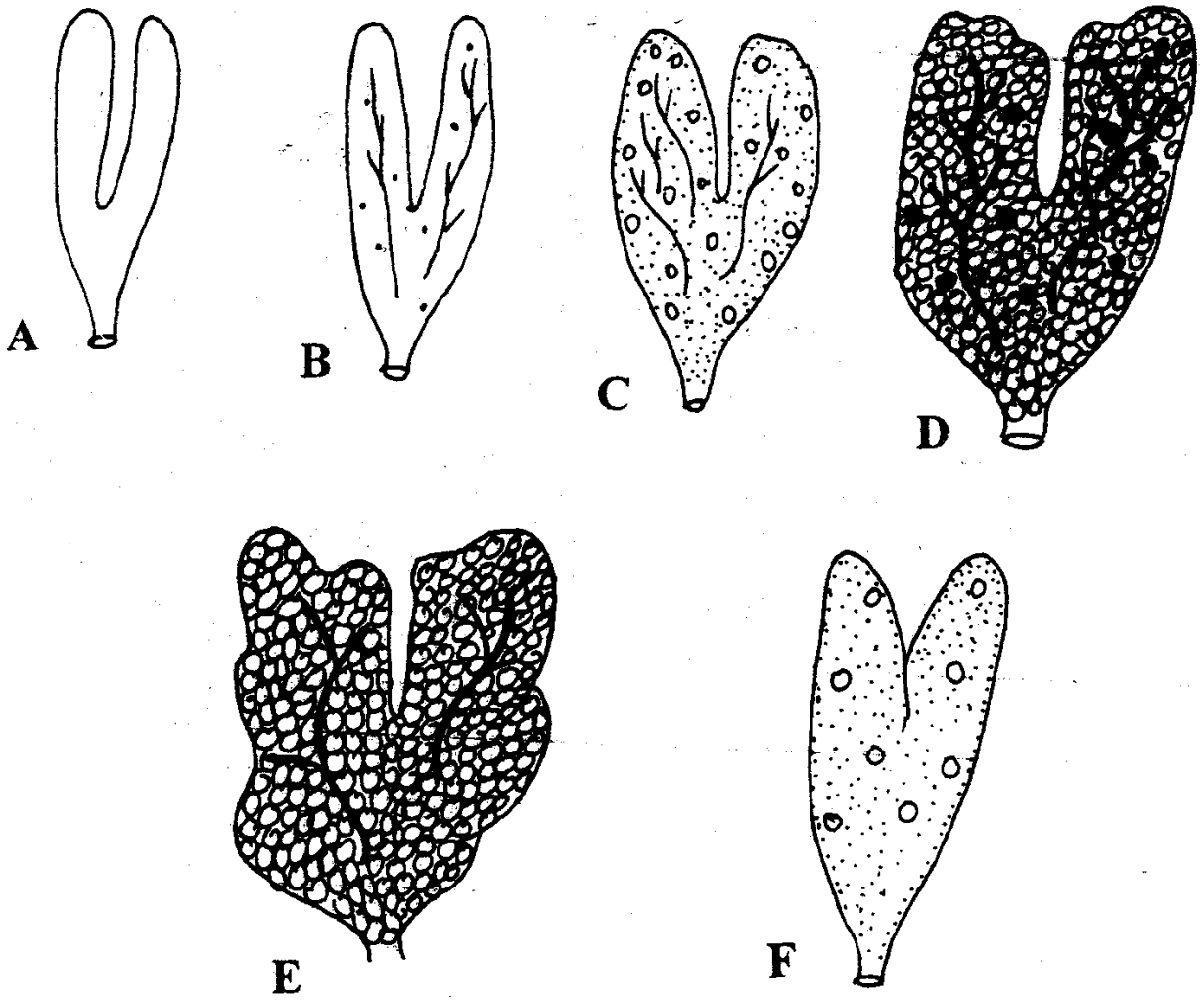
2. Preparatory phase: oocytes are in stage III. There is beginning of **vitellogenesis** in the oocyte cytoplasm. Oocyte envelops get differentiated with the thecal and granulosa layers assuming the steroidogenic function. Cells of granulosa layer **start producing female hormone, estradiol**.

3. Maturing or Prespawning phase: Characterized by intensive process of vitellogenesis by which ooplasm of an oocyte is loaded with yolk granules and in stage IV. **1st meiotic division completed and second meiotic division occures.** The size of the ovary and GSI will be maximum at this phase.

4. Spawning Phase: characterized by gravid ovary containing ripe oocyts. During spawning follicle of fully ripe oocytes rupture as a result oocytes released into ovocoel (cystovarian ovary) or body cavity (gymnovarian ovary) from where they pass out through genital pore into water.

5. Post spawning phase: the ovary exhibits a collapsed appearance as evaculated follicles are seen after the release of eggs.

Seasonal Changes in the ovary of teleost



Seasonal changes in the ovary of a teleost; **A** Resting phase, **B** Early maturing, **C** Advanced maturing, **D** Mature, **E** Spawning phase, **F** Spent phase.

Vitellogenesis

Accumulation of yolk substances in eggs called vitellogenesis

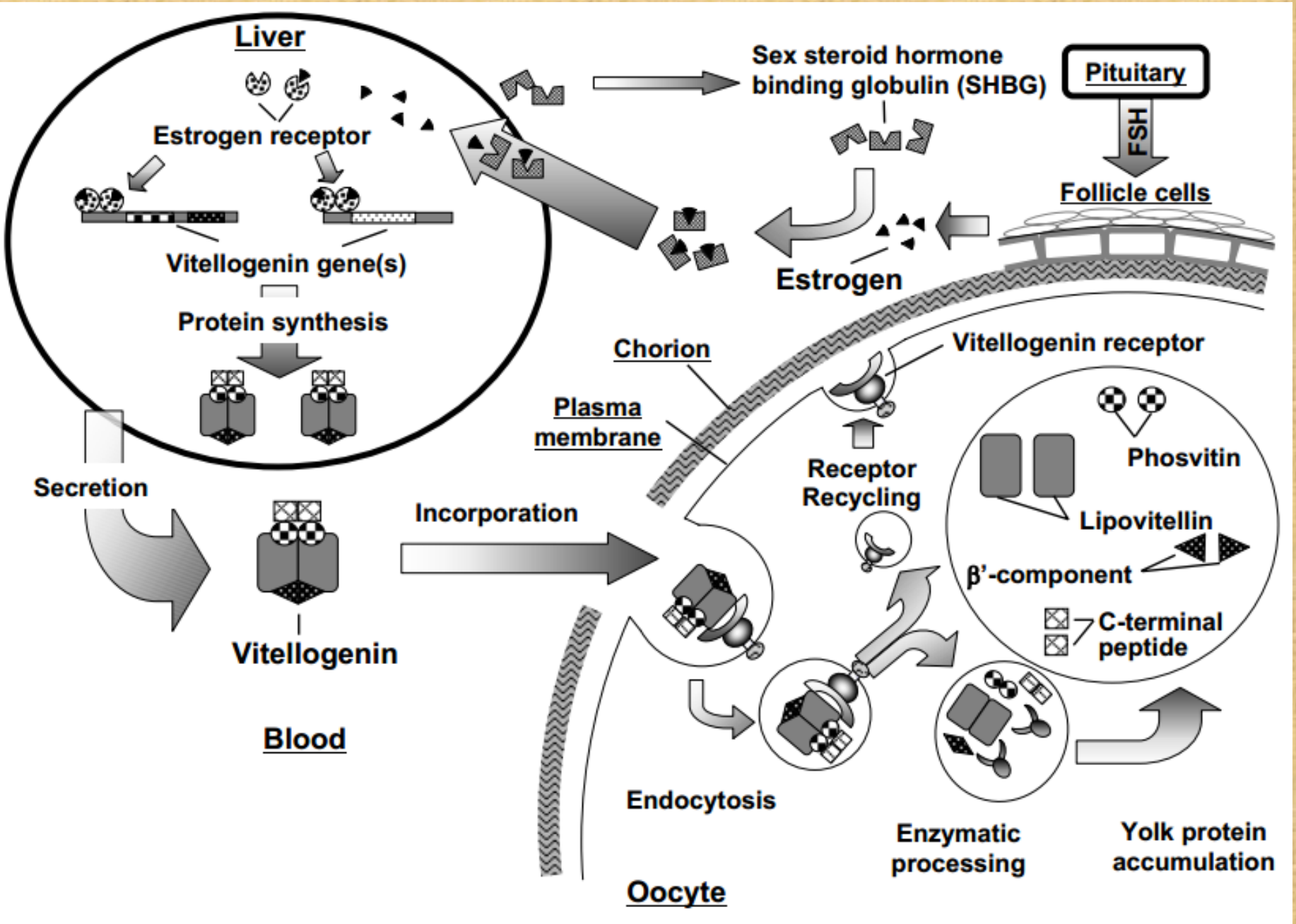
At the beginning there is no yolk. Blood of mature female contain a calcium binding lipophospoprotein called vitellogenin which is synthesized in the liver under the stimulation of female sex steroids. It passes from the plasma to oocyte and undergoes limited proteolysis give rise to yolk protein lipovitellin (Lv), phosvitin (Pv), β'-component (β'-c), and C-terminal peptide.

Yolk protein deposited as three yolk substances:

Yolk vesicles -contain glycoprotein
yolk globules-lipoprotein
oil droplets- Glycerides

After completion of vitellogenesis, movement of germinal vesicles to animal pole, Fusion of yolk granules and grouping of oil droplets occurs

Vitellogenesis



Gonadosomatic Index (GSI)

The Gonatosomatic index of a species can be calculated by the following formula

GSI= Weight of the gonads/weight of the fishX100

Gonadosomatic index of a species has been widely used to indicate the maturity and periodicity of the spawning of the fish. The GSI increases with the maturation of the fish and is maximum during the peak period of maturity. It decrease abruptly after spawning.

Fecundity

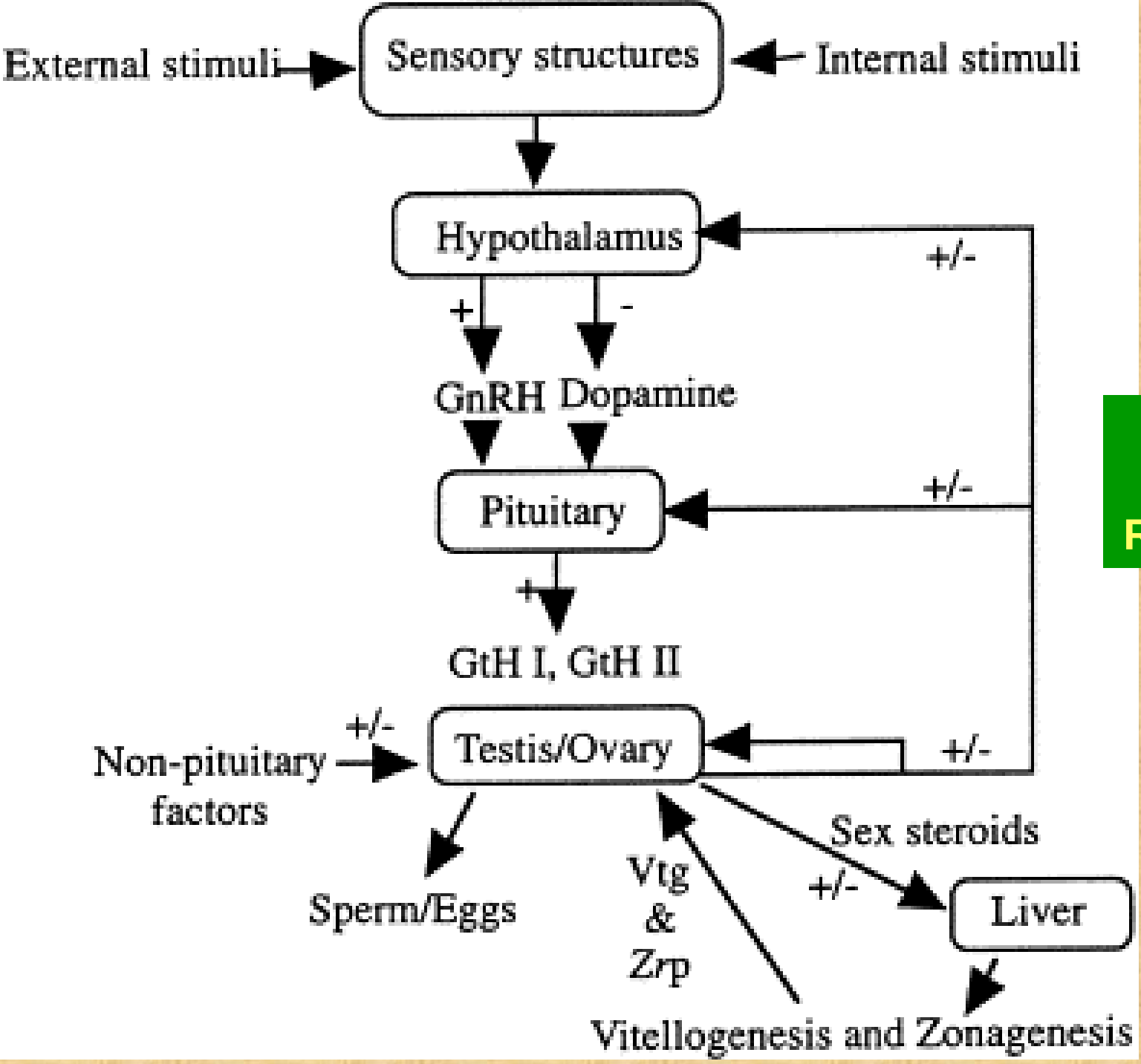
Fecundity is a measure of the reproductive capacity of a female fish and can be defined as **the number of ova that are likely to be laid by a fish during the spawning season**. It varies from species to species and different individual of same species may also exhibit variation depending upon size, age, nutritional status, environmental factors and genetic composition.

The fecundity of a species can be measured by any one of the following

(i) Volumetric Method: The mature ovaries are taken out of the abdomen and total volume is determined. Now small pieces of the ovary are taken as random samples from anterior, middle and posterior parts. The volume of each sample is determined and number of ova in each is counted under a lens. The total number of ova in the total volume of the ovaries is then calculated.

(ii) Gravimetric Method: mature ovaries are preserved in 10% formalin for estimating fecundity. Weight of the ovaries is determined. And three samples of 100 mg each are taken at random from anterior, middle and posterior parts. The the number of ova in each sample are counted under a binocular microscope. Tthe total volume of the ova are calculated as follows

Fecundity=average number of ova from three samples of 100 mg each X total weight of ovary/100



Hormonal Control of Reproduction

Embryonic Development

Fish embryonic development consists of seven stages leading to hatching. These stages are the **zygote period**, **cleavage period**, **blastula period**, **gastrula period**, **segmentation period**, **pharyngula period**, and **finally hatching**.

Zygote period: Soon after fertilization, the micropyle is closed thereby preventing the entry of any other sperm into oocyte. The perivetelline space gets filled with water and colloid, swelling of oocytes takes place. This phenomenon is called water hardening. During water hardening the animal pole develops a small hillock of protoplasm on yolk mass known as egg blastodisc.

Cleavage period: Cleavage furrow is limited to cytoplasm of animal and such type of cleavage is called meroblastic cleavage. As successive cleavage continue, a group of cells are formed and it is called morula.

Blastula period: After further division the cell arrange in to the from of a layer called blastoderm. Each cell of blastoderm is called blastomere. Cleavage also result in the formation of another type of cells called periblast which lie between blastoderm and yolk. The embryo develops from the blastoderm. A space develop between the blastoderm and periblast which is called segmental cavity or blastocoel. At this stage embryo is called blastula. At blastula stage presumptive germ layers (ectoderm, endoderm and mesoderm) can be noted.

Embryonic Development continued…….

Gastrula period: The process of formation of gastrula is called gastrulation. At this time the under rim of blastodisc thickens to form a merginal ridge and inner layer of this is called germ ring. The germ ring is thickest at the caudal end, which is called embryonic shield. Gastrulation occurs by three process invagination, involution and epiboly. An invagination appears at the future endodermal region of blastula . The portion that is invaginated grow below the blastoderm and this portion is called hypoblast. The cells of endoderm and mesoderm multiply and migrate inward over the lip of blastopore and this process is called involution. During this process the cells of the blastoderm also continue to grow over the yolk which is called epiboly. As a result yolk mass is covered by a layer of cells called epiblast and future ectodermal cells are involve in this process. The periblast also grows over the yolk forming the inner layer covering the yolk below epiblast. Inner priblast and outer epiblast layer enclose the yolk which is called yolk sac. With the formation of yolk sac gastrulation is completed.

Segmentation period: The primary organ rudiments appear in the anterior part of the embryo. Anterior part of the neural plate from where the brain develops appears first. Notochord gets separated from mesoderm. Mesoderm laying on the both side of notochord gets segmented and various organs of body are formed. This result in a small embryo with cylindrical and bilaterally symmetrical body.

Embryonic Development continued…….

Pharyngula period: In this stage embryo will begin to exhibit fully formed organs Ectoderm give rise to epidermis and its derivative like enamel of teeth, olfactory epithilium, lens of eye, inner ear, brain, spinal cord, retina and optic nerve. Mesoderm divide into three parts the dorsal epimere, middle mesomere and ventral hypomere. Epimere becomes divided in to somites, each somites having three parts such as outer dermatome, middle myotome and inner sclerotome. Dermatome give rise to connective tissue and muscles of dermis of skin, myotome give rise to muscle of trunk, appendicular skeleton, fins and their muscles. Vertebral column is derived from sclerotome. Kidney, gonads and their ducts are derivatives of mesomere. Hypomere develops to somatic and splanchnic layers of mesoderm and enclose coelomic cavity. Mesenchyme cells derived from splanchnic layer develops to involuntary muscles and connective tissue of gut, head and blood vessels. Mesenchyme of head region gives rise to skeleton and muscle of head and outer layer of eye. At the end of this stage embryo become active and exhibit continuous twitching movement which show that embryonic development id completed.

Embryonic Development continued…….

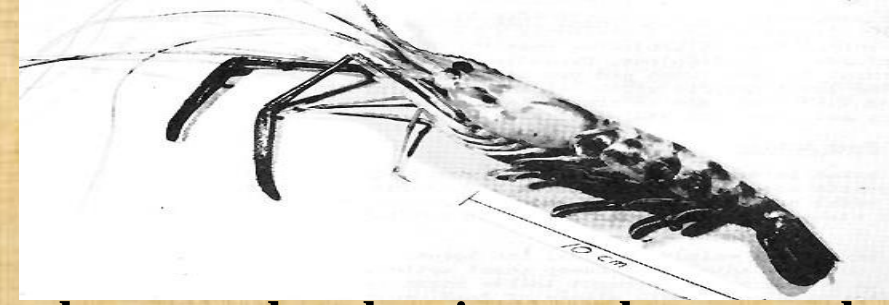
Hatching: There are two mechanism involve in hatching 1) mechanical and 2) enzymatic. In mechanical hatching egg envelops are broken down by mechanical action such as pressure excreted from within or mastication by embryo itself. In enzymatic hatching, emergence of young occurs after dissolution or softening of egg envelope by the enzyme secreted by embryo. This enzyme is called hatching enzyme. These enzymes are secreted into perivitelline fluids at the time of hatching from hatching gland cells located in the epidermis of the embryo.

Reproduction in Freshwater Prawn

Sexual Dimorphism

The sexes are separate and there is well marked sexual dimorphism in fresh water prawn ***Macrobrachium rosenbergii***

Male	Female
•Larger, 2nd pereopod much larger and thicker.	•Smaller, 2nd pereopod thinner and shorter.
•Cephalothorax larger, abdomen narrower.	•Cephalothorax smaller, abdomen broader.
•Appendix masculina and appendix interna at endopodite of 2nd pleopod.	•Appendix masculina is absent.
•Genital pores at the base of 5th walking leg.	•Genital pores at the base of 3rd walking leg.
•Pleura of abdomen is shorter.	•Pleura of 1st, 2nd, 3rd abdominal segment are longer and forms a brood chamber
•Reproductive setae are absent.	•Reproductive setae present.
•Distance/gap between last pair walking leg is very less in male.	•Distance/gap between last pair walking leg is more in female



•Ventral side of the abdominal segment has a lump- a hard point- at the centre which can be felt with finger.

•No lump or hard point at the ventral side of 1st abdominal segment.

•Reproductive setae are absent on the ventral side of thorax and pleopods of mature male.

•Reproductive setae appear on the ventral side of thorax and pleopods of mature females such as ovipositing and ovigarous setae. Ovipositinng setae appears on coxa of last 3 pairs of preopods and posteriors margin of sperm receptacle area and the pleopods. It helps in guiding and propelling eggs during spawning. Ovigarous setae serve to anchors the eggs to pleopods.

•No sperm receptacle in the thoracic sterna between last three pairs of preopods.

•There is an sperm receptacle on the between last three pairs of preopods.

Peak Breeding seasons

Hoogly estuary – March to May

Kerala backwater- Oct and Nov

AndhraPradesh- Aug to Oct

Reproductive System

Male

1) **A pair of Testis** located on mid dorsal side of cephalothorax below carapace

2) **Vas deferens** has 4 regions

- Proximal region
- Medial region
- Distal region and
- Terminal ampoule which open at the base of the coxa of 5th pereiopod

Female

1) **A pair of ovaries** located dorsal to the stomach and hepato-pancreas in the cephalothorax cavity and Each ovary consist of anterior lobe, lateral lobe and abdominal lobe

2) **Oviduct** which open at the base of the coxa of 3rd pereopod

4 stages of ovary

1. Immature
2. Early maturing
3. Maturing
4. Ripe

Different stages of ovary

Stage	colour	Oocytes size (mm)	Ovary size & position
Immature	transparent	0.064 to 0.128 Spherical	Vary and present in posterior most region of carapace cavity
Early maturing	Yellowish due to light deposition of yolk	0.191 to 0.447	Occupy ¼ to ½ of total carapace cavity
Maturing	Light orange in color due to heavier deposition of yolk	0.319 to 0.547	¾ of carapace cavity
Ripe	Dark orange Ova become opaque due to heavy yolk	0.4468 to 0.7761	Occupy entire carapace cavity

Mating

- **Take place between hard shelled males and mature soft shelled females which have just completed their premating moult.**
- **Time between moulting and mating varies from 1.2 to 21.8 hrs (avg. 9.1 hrs).**
- **Male gonopore brought close to sperm receptacle of female on thoracic region and transfer the sperm to receptacle as spermatophore. Spermatophore adheres to the sperm receptacle because of the glutinous nature of attachment matrix**
- **After mating female may spend several hours in prespawning preening behavior.**

Spawning

- Spawning occurs after few hrs of mating.
- Eggs laid by female are fertilized by sperm attached to underside of thorax.
- The fertilized eggs are transferred to the ventral side of abdomen (brood chamber) by ovipository setae and attched to the ovigerous setae of 1st 4 pleopods and kept in place by a membrane.
- Movements of pleopods helps in aerating the eggs.
- Adult females carrying eggs on the ventral side of abdomen is called berried female.
- Fecundity varies from 20000-100000 eggs. However, their first broods, (i.e. those which are produced within their first year of life), are often not more than 5 000 to 20 000.

Incubation

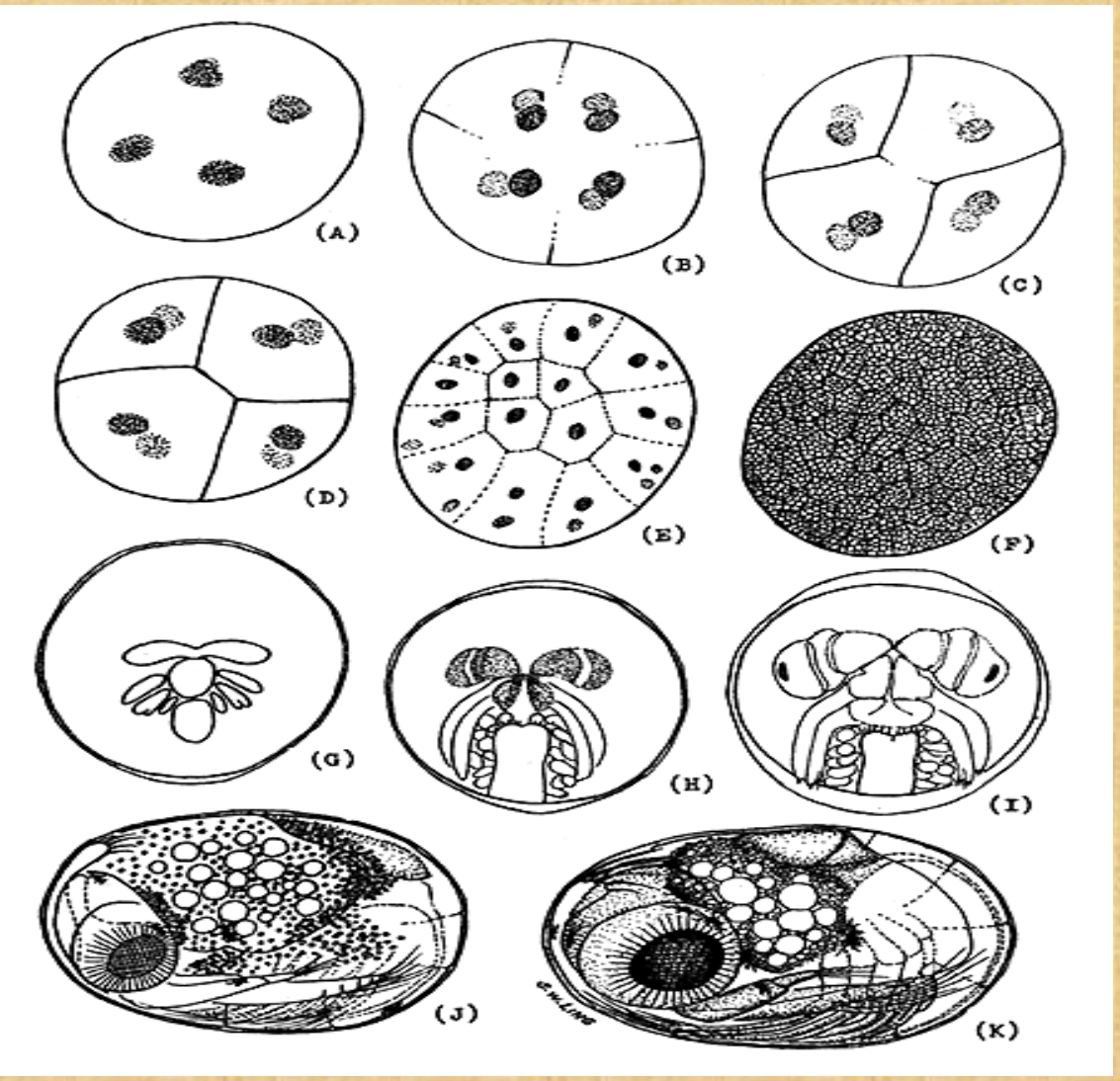
•Initially eggs are slightly elliptical (0.6-0.7 mm in long axis) and bright orange in color

•Embryonic development proceeds.

• Incubation period avg. 19 days.

• Turns grey black 2-3 days before hatching.

• Freshly hatched larvae are called zoea.



Macrobrachium rosenbergii, segmentation and embryonic development.

Times refer to period since fertilization.

(A) 7 h - completion of **second nuclear division**. **(B)** 8 h 45 min - **third nuclear division** nearly completed, appearance of **4 cleavage furrows**. **(C)** 8 h 55 min - third nuclear division completed, tips of the 4 cleavage furrows have met at 2 points from which the **median furrow is developing. (D)** 9h - complete formation of 4 quadrants **(blastomeres**). **(E)** 14 h - **32 nuclei**. **(F)** 24 h - completion of **segmentation**. **(G)** 6 days-formation of **caudal papilla**. **(H)** 7 days-formation of **optic vesicle**. **(I)** 9 days-**eye pigment** developed. **(J)** 14 days **-larva fully formed**. **(K)** 19 days-**larva ready to hatch**.

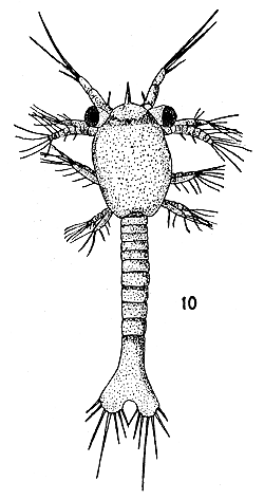
Development of Larvae

Newly hatched zoea about 1.92mm in length. It has the zoeal characteristics such as a body distinguishable into cephalothorax and abdomen.The segmented body is without pleopods. Larvae swim upside down by using their thoracic appendages and are positively attracted to light.

By stage XI they are about 7.7 mm long. Newly metamorphosed post larvae (PL) are also about 7.7 mm long and are characterized by the fact that they move and swim in the same way as adult prawns. They are generally translucent and have a light orange-pink head area.

Larvae require salinity from 11-13 ppt for survival and growth.

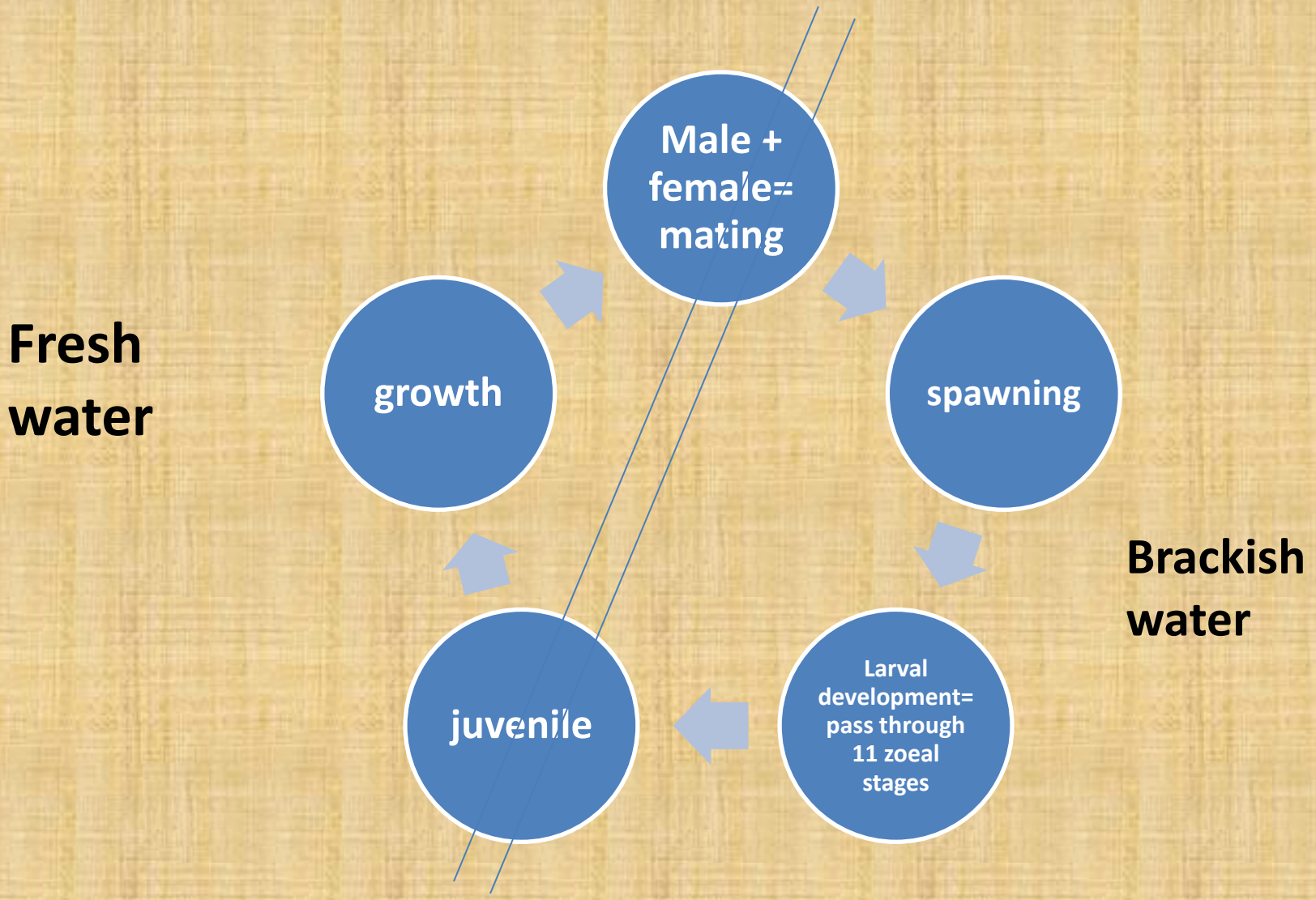
Larval passes through 11 zoeal stages within 23-32 days depending on temperature, food, and water quality to become PL.



Identifying Characters of larval stages

Larval stage	Age (days)	Total length (mm)	Distinguishing features
I	1	1.92	Sessile eyes
II	2	1.99	Stalked eyes
III	3	2.14	Uropod present
IV	4-6	2.5	Two dorsal rostral teeth, uropod biramous with setae
V	5-8	2.8	Telson narrow, elongated
VI	7-10	3.75	Pleopod buds appear
VII	11-17	4.06	Pleopods biramous and bare
VIII	14-19	4.68	Pleopods with setae
IX	15-22	6.07	Endopods of pleopods with appendix interna
X	17-24	7.05	3-4 dorsal rostral teeth
XI	19-29	7.73	Teeth on half of upper dorsal margin
PL	23-27	7.69	Teeth on upper and lower margin of rostrum, adult behaviour

Life cycle


Male + female= mating
spawning
Larval development= pass through 11 zoeal stages
juvenile
growth
Fresh water
Brackish water