Reproduction - Major Issues

Gametogenesis - The Process
Gametogenesis – Control Over Process
Ovulation, Spawning and Fertilization
Growth of the Embryo – Life Cycle Stages
Growth of Juvenile and Adult
Somatogenesis vs. Gametogenesis
Getting Seed for Aquaculture
Reproduction & Aquaculture

Farms Need Seed to Function - Options:
Can Buy Seed: Cost can Be Considerable
Can Culture Seed: Need Specialty Facilities
Can Grow Seed Naturally: Need Control Over
  Oocyte Maturation
  Fertilization
  Larval Feeds
  Metamorphosis

What is the Best Option for Your Farm?
Gametogenesis – The Process

In the Developing embryo

Primordial Germ Cells (PGCs)

PGCs migrate to developing gonad

Period of mitotic proliferation

Undergo meiosis I and meiosis II

Differentiate into mature gametes

Either ova or spermatozoa
Some Cell Biology Background

Reference:

Covers Advances in Molecular Physiology
Useful to Consider Gametogenesis in Context of The Cell Cycle.

See Egg as Provisioned to Support Embryogenesis
Cell Cycle Phases

\[ G_1 \] – Growth 1 – Cytoplasmic Synthesis
\[ G_0 \] – Quiescence After or In Place of \( G_1 \)
\[ S \] – DNA Replication \( 2c \rightarrow 4c \)
\[ G_2 \] – Growth 2 – After DNA Replication
\[ M \] – Mitosis – Cell Division

\( n \), number of gamete chromosomes,
\( c \), amount of gamete DNA
Cell Cycle

- **G₁**: Synthesis of components required for DNA synthesis (40%)
- **S**: DNA synthesis (39%)
- **G₂**: Preparation for mitosis (19%)
- **M**: Mitosis and cell division (2%)
- **G₀**: Quiescence (variable)
Metazoan Gametes

Spermatozoan
  Specialized for Motility and Membrane Fusion
  Highly Adapted to Deliver DNA to the Ovum

Ovum
  G2 Arrested - Prolific Synthesis Ongoing
  Maternal Messenger mRNA, Mitochondria etc
  Yolk & Cytoplasm to Support Embryogenesis
Meiosis

Turn a $2n4c$ $G_2$ Arrested Cell $\rightarrow$ into a $1n1c$ Haploid Gamete in 2 Steps

Step 1. $2n4c \rightarrow 2n2c$ (Ordinary Cell Division)

Step 2. No S Phase – no DNA Replication

each $2n2c \rightarrow 2 \ (1n1c)$ or

each $2n2c \rightarrow 1n1c$ and a Polar Body
Starfish Gonadogenesis

Annual Growth of Starfish Gonads
Dependent on Day/Night (Light/Dark) Ratio
Experiment – Manipulate Day Night Ratio
To Half a Year Later - over 4 Months.
Start in June – by September
Light Regime Equaled March
Result: Entrained Experimental Starfish
To New Light/Dark Schedule – Showing
Environment Control Over Reproduction
Caution: 2 Minutes Light in Dark Period
Would Throw Experiment Off
Spermatogenesis

PGC in ♂ Gonad Called Spermatogonium
Spermatogonia are Diploid Cells (2n4c)
Proliferate by Mitosis in Testis
Prophase Meiosis I – Now 1° Spermatocytes
Meiotic Division I – 2° Spermatocytes
Meiotic Division II – Spermatids
Differentiation – Mature Sperm
4 Sperm per Primary Spermatocyte
57 Cell Divisions from Zygote to Sperm
Spermatogenesis

Spermatogonium (2n) → Mitosis → Primary Spermatocyte (2n) → Meiosis I → Secondary Spermatocyte (1n) → Meiosis II → Spermatid (1n) → Sperm (1n)
Oogenesis

PGC in ♀ Gonad Called Oogonium
Oogonia are Diploid Cells (2n4c)
Proliferate by Mitosis in Ovary
Prophase Meiosis I – Now 1° Oocytes
1° Oocytes in a G₂ Arrest – Massive Synthesis Stimulated by Maturation Hormone
Meiotic Division I - 2° Oocyte and Polar Body
Meiotic Division II - Ova and Polar Body
27 Cell Divisions Between Zygote & Ova
Oogenesis
Gametes

*Ovum* – The Largest Cell in the Animal
Salmon Egg, diam. ~ 3 mm or 3,000 μm, 78 x 10⁶ times volume of Salmon Red Blood Cell, diam. ~ 7 μm

*Sperm* - Head Highly Condensed Chromosomes
Almost no Cytoplasm – Midpiece – Mitochondria
Tail – 9+2 Flagellum, Motion is Ca⁺² Activated
Gametes

Isogamy: Gametes of Equal Size, Have Mating Types not Sexes

Anisogamy: or Heterogamy – Gametes Different Sizes
Male is Defined as the Smaller Gamete

Oogamy: Extreme Heterogamy – Gamete Specialization
Ovum – Specialized for Cell Contents, Non-Motile
Sperm – Specialized for Motility, Minimal Cytoplasm
Isogamy

Motile

Non-Motile

Conjugation
Anisogamy

A

Both Motile

B

Oogamy

C

Both Non-Motile
Animal Life Cycle Model

♂ and ♀ Haploid (1n) Gametes

Spawning (Ovulation)

Gametes Fuse at Fertilization

Fertilization Produces Diploid (2n) Embryo

Development Through Stages

Reproductive Adult – Gametogenesis

Produces Haploid Gametes
Plant Model Can Be Relatively Complex

+ and – Haploid (1n) Gametes,
Gametes Fuse in Fertilization
Fertilization Produces Diploid (2n) Embryo
Embryo Grows by Mitosis
Becomes a Diploid *Sporophyte*,
Sporophyte Meiosis to Produce
Haploid (1n) Spores,
These Grow by Mitosis
Become a Haploid (1n) *Gametophyte*
Grows by Mitosis Producing Haploid Gametes
Control Over Ovulation in Starfish

Starfish Radial Nerves Contain a Hormone Called Gonad Stimulating Substance \textit{GSS}. Applied to Isolated Starfish Ovaries it Induces the Maturation Inducing Substance (\textit{MIS}). Identified as 1- methyladenine (1-MA). Synthetic 1-MA Caused Starfish Oocytes to Undergo Meiosis and Become Fertilizable.

Time for GSS Action – 4 – 12 hours

Time for MIS Action – 45 min to 4 hours
Starfish Oocyte
Starfish Oocyte Maturation
Meiotic Divisions
Oocyte Maturation & Spawning

Eggs Spawned as 2\textsuperscript{nd} Polar Body is Forming
Prior to this Sperm Cannot Fertilize Egg
Synchronize ♂ and ♀ Spawning
~5 Hours or More for GSS & MIS to Work
Vertebrates Use Different Hormones
Successful Fertilization within 15 min of Spawn
Dilution of Sperm & Eggs, Sperm Incapacitated
Starfish Spawning Posture
Crown of Thorns Starfish Spawning
Animal Life Cycle

- Fertilization
- Cleavage Stage
- Blastula
- Mid Blastula Transition
- Hatching
- Gastrulation
- Larvae
- Metamorphosis
- Adult
- Gametogenesis
Fertilization

Fusion of Sperm Plasma Membrane With Plasma Membrane of Ovum

Consequences

Electronics of Fertilization Reaction

Fertilization Cortical Response

Formation of Fertilization Envelope

Function of Fish Egg Micropyle
Starfish Mechanical Block to Polyspermy
Electronic Block to Polyspermy

(a) Images showing the process over time:
- 55 s: Early stage
- 85 s: Mid-stage
- 115 s: Further progression
- 145 s: Mid-stage
- 175 s: Late stage

(b) Diagram explaining the process:
- Activated sperm binds to the egg surface
- Vitelline envelope around egg
- Egg plasma membrane
- Cortical granule releases its contents
- Cortical granule contents cause creation of fertilization envelope
- Sperm fuses with the egg

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Fertilization - Calcium Wave
Polyspermy

Fertilization by More than 1 Sperm

Triangular Mitotic Apparatus

Fatal - Tears Chromosomes Apart

Blocks to Polyspermy

Electronic Block from Internal Ca$^{+2}$ Stores

Fertilization Membrane (Envelope) from Material in Exocytosed Cortical Granules
SEM Polyspermy
Fertilization Envelope Blocks Polyspermy
Cleavage Stage

Function: Reducing Nuclear Cytoplasmic Ratio

No mRNA Transcription from Fertilization Until Mid-Blastula Stage

Embryonic Genome ‘Turns On’ at Mid-Blastula

Until that time all Protein Synthesis from Stored Maternal mRNA
Starfish - Synchronous Cleavage
4 Cell Starfish Cleavage Stage Embryo

Fertilization Envelope Also Visible
Development of Germ Layers

Blastula – A Hollow Ball Undifferentiated Cells

Mid-Blastula Transition
  Before – all mRNA from Maternal Stores
  After – new mRNA from Embryonic Genome

Gastrula – Development of the 3 Embryonic Tissues – Ectoderm, Endoderm, Mesoderm
Blastula & Gastrula

- Blastula
- Gastrula
- Blastopore
- 8-cell stage
- Single Cell
- Larva
- Gastrula
$r & K$ Selection

$K$ (carrying capacity) Selected
Few Eggs, Extensive Yolk, Large Size, Brooders
Nest Builders, Guard Eggs, 90% Survivorship
Larvae: Lecithotrophic and Direct Developers

$r$ (maximum growth rate) Selected
Many Eggs, Little Yolk, Small Size
Broadcast Spawners, 5-10% Survivorship is High
Larvae: Planktrotrophic, Indirect Developers

$K$: FW Shrimp, $3 \times 10^4$ eggs; $r$: Penaeid, $2 \times 10^6$ eggs
Larval Forms

Planktotrophic – Feeding in the Plankton
Lecithotrophic – Feeding on Sequestered Yolk

Direct Development – Directly Assumes Adult Body Plan & Morphology

Indirect Development – Have Other Body Plans & Metamorphosis (profound tissue rearrangements) Before Assuming Adult Form
Planktotrophic Larva

Pluteus Larva
From Sea Urchin
Ciliary Bands for Feeding & Locomotion
Lecithotrophia embryo

Frog

Xenopus
Starfish Direct Development
Starfish Indirect Development
Brachiolaria Larva
Starfish Metamorphosis

Adult Will Develop From The Pigmented Mass Larval Tissues Resorbed
Insect Metamorphosis

Complete Metamorphosis:
Life Cycle of the Lady Beetle

Larva
Eggs
Pupa
Adult

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http://aggie-horticulture.tamu.edu/galveston
Amphibian Metamorphosis

- Eggs
- Embryo inside egg
- Gills are outside
- Tadpoles
- Skin grows over gills
- Hind legs form
- Tail becomes shorter
- Adult
Juveniles

Organism has Achieved the Adult Body Plan

Gonad Differentiated but Still Growing (Mitosis)

Often Takes Several Years for 1st Reproduction

Species Specific Points

Age 1st Reproduction  Sea Cucumber 5 years
Egg Diameter –       Sea Cucumber 180 μm
Eggs per Year –      Sea Cucumber 8x10^6
Aquaculture - Closing The Life Cycle

Purchase Fertilized Eggs (Embryos)
Feed & Raise Embryos to Juvenile Stage
Feed & Grow Juvenile Fish into Adults
Isolate Eggs and Sperm from Broodstock
Mix Together for *in vitro* Fertilization.
Raise Embryos to Juveniles
Grow Juveniles to Adults.
Knowledge and Technologies Required to Maintain Entire Life Cycle of the Species

- Allows Initiation of Reproduction
- Allows Control over Reproduction
- Allows Selective Breeding Programs
- Allows Genetic Manipulations
- Allows Detailing Nutritional Physiology
- Allows Defining Immune Function

Aim is Domestication of Species
Carp Breeding I

Strains Generations Removed from Wild.
Carp Can Breed Throughout the Year
Carp Seasonal Breeding – Spring Warm-Up
Spawning After Dark or In Morning
Segregate Sexes to prevent Spawnings.
Eggs laid on Foliage near Water Surface
Carp Breeding II

Spawning Stimulated by Transfer to Shallow Spawning Ponds
Fibrous Materials Substitutes for Foliage
Tropical Carp Cab Breed 2-4 times / yr
Temperate Zones - Pituitary Gland Extracts Contain Gonadotrophic Hormones Used to Provoke Natural Spawning or Aid in Stripping Gametes.
Fertilized Spawn Moved to Hatching Ponds
Pituitary Gland Hormones

Luteinizing Hormone (LH) in Both ♂ & ♀
In ♀ LH Stimulates Ovary to Produce Progesterone Directs Ovulation & Corpus Luteum Formation
In ♂ LH Stimulates Testis to Produce Testosterone
LH is a Glyco-Protein Hormone with α and β Chains
Gonad Target Tissue Produces Steroid Hormone
LH Acts Like Starfish GSS, the Steroids Like MIS
Carp Eggs in Zoug Jars
Fish Egg Micropyle

Micropyle is sperm entry point
In Vitro Fish Fertilization
Fertilizing Salmon Eggs
Salmon Life History Stages

Zygote (Fertilized Egg)
Eyed Embryo
Alevin
Parr
Smolt
Smoltification
Eyed Embryos
Atlantic Salmon Alevin (Yolk Sac)
Chum Salmon Parr
Salmon Smolt
Smoltification

Suite of Profound Physiological, Morphological Biochemical and Behavioral Changes in Salmon Parr that Take Place as They Prepare to Migrate Downstream and Enter the Sea.

Smoltification: What Makes Salmon Anadromous
Fish Development to Hatching
Carp Hatching and Rearing

Eggs prone to fungal infections, treat with fungicide - or drying out hatching ponds.

Hatched carp cement themselves to substratum for up to a week while absorbing yolk sac.

Hatchlings plus 1 week need to feed – primary foods zooplankton or phytoplankton.

Most vulnerable from hatching to 3 gms.

Need to be cautious with density.

Swimming juveniles moved to nursery ponds.
Carp Hatching

a. hatched larva with yolk sac
b. Embryo preparing to hatch
Catfish Life Cycle

Catfish Eggs
Mouth Brooding Tilapia
Catfish Life Cycle

Just Hatched
Catfish Life Cycle

Juvenile Striped Catfish – *Plotosus lineatus*
Tilapia Fertilizing Ponds

Main Objective – Stimulate Primary Productivity

Main Inputs: Nitrogen, Carbon & Phosphorus

Optimal Ratio: 45-50% C…
   8-10% N
   1% P

Organic Fertilizers:

Poultry Manure  Domestic Sewage
Cattle Manure  Green Manure
Composted Agricultural Waste
Larval Fish Feed - Zooplankton

Gravid Copepod
Juvenile Fish Feed

Volvox
Adult Fish Feed

Melosira