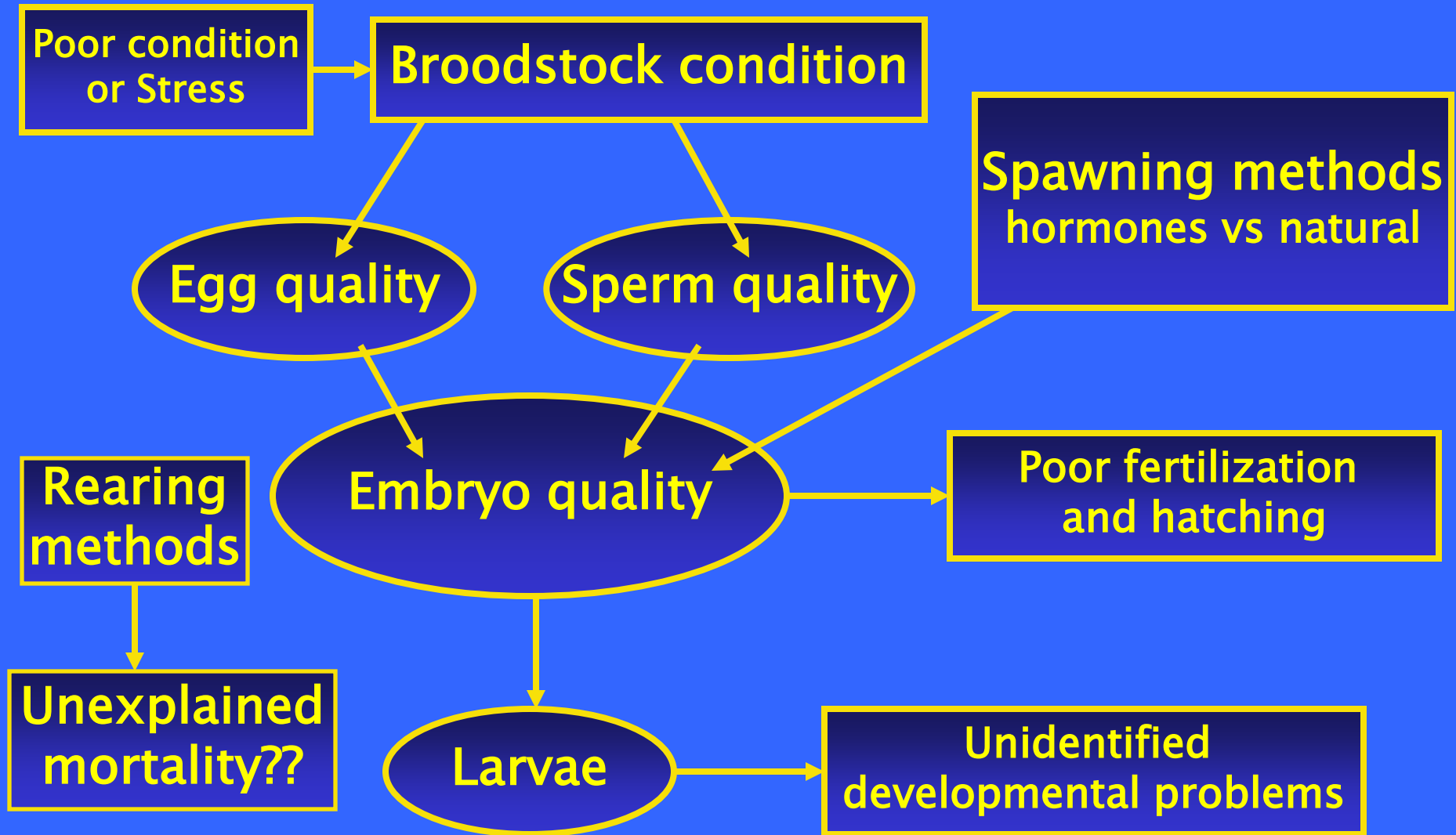
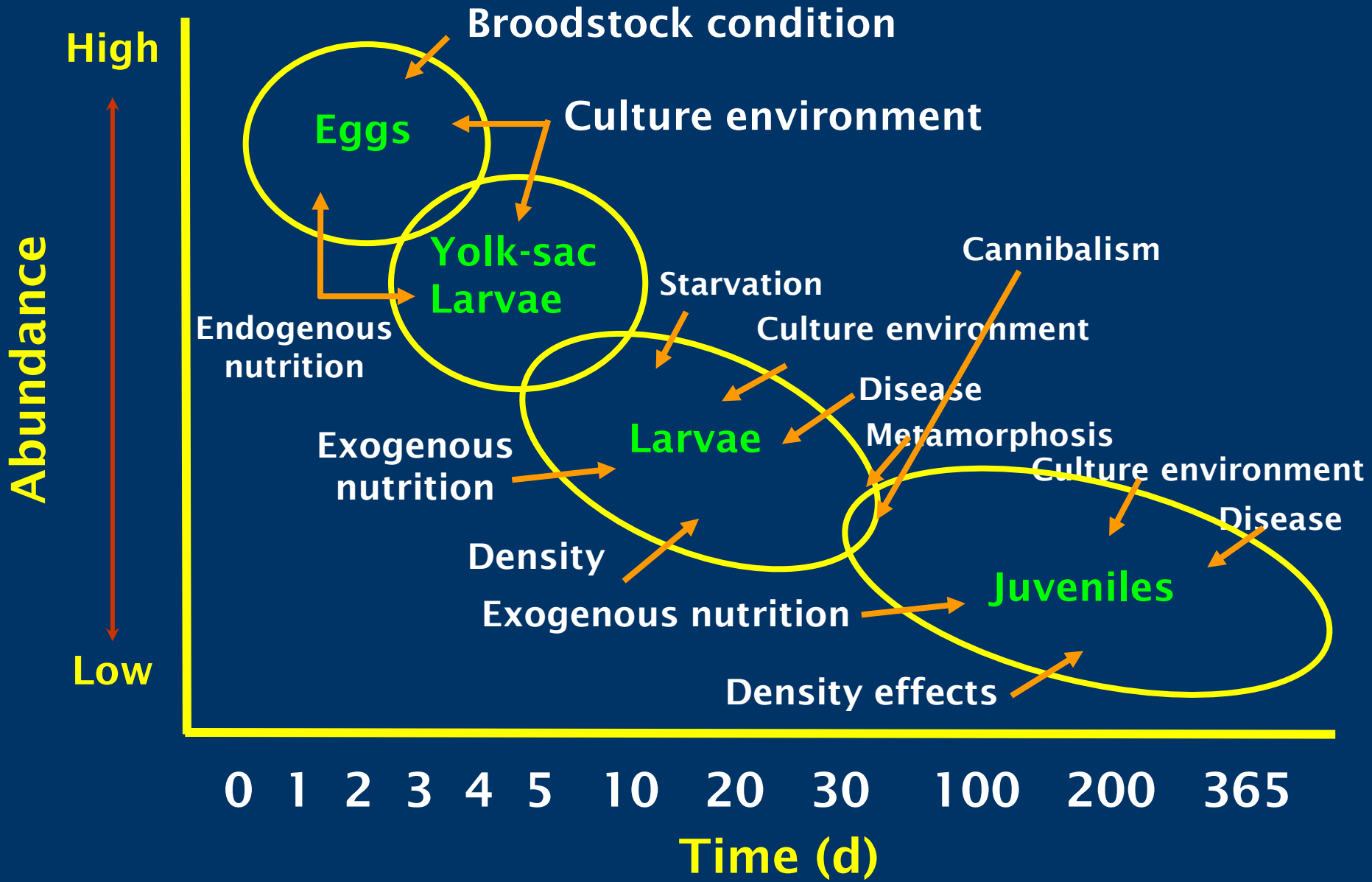


The background of the slide is a microscopic image showing several fish eggs and early-stage larvae. The eggs are roughly spherical with a distinct outer layer and a darker central spot. The larvae are elongated, with visible heads and tails, and some show developing fins. The overall color palette is warm, with yellows, oranges, and browns.

Pompano Broodstock Maturation and Larviculture Protocols

Potential Bottlenecks in the Production of Fish Larvae





Broodstock

- Quarantine
- Maturation System Design/Stocking
- Feeding
- Conditioning
- Spawning/Hormone manipulation

Quarantine

- All new fish brought into the facility are isolated until cleared of any and all parasites
- This can take up to several weeks
- Disease/acclimation procedures
- Weening/acclimation procedures

Disease/Acclimation Procedures

- Pompano will always come in with capture wounds
- Immediately treat with a freshwater dip for 5 minutes
- After one week, a prophylactic treatment of formalin (250ppm for 1 hour)
- If disease event occurs after this, determine disease agent and treat accordingly (e.g., Amylo- .20-.25ppm Cu for 3 weeks)

Weening/Acclimation Procedures

- Pompano will typically not eat for the first week in captivity
- To get them started, use frozen krill
- Ultimately, you want to get them on the maturation diet food mix

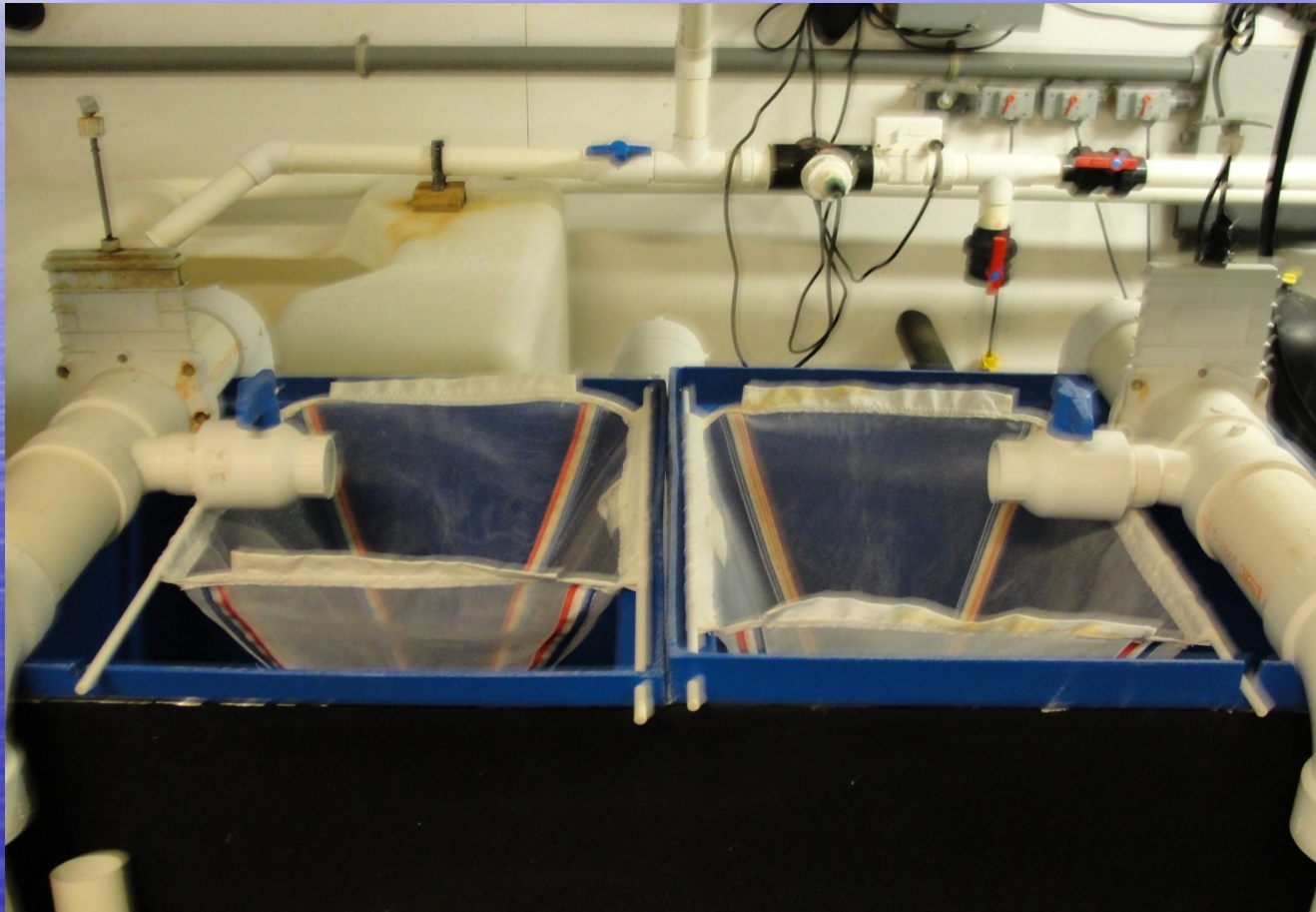
System Design

- Tanks should be supplied with own air/LOX and water inlets so that individual tanks can be isolated if chemical treatments are necessary
- Recirculating System Components:
 - Biofilter
 - Cartridge filtration
 - UV sterilization
 - Temperature control (heat pump)
 - Lighting control

Broodroom System



Broodroom System



Stocking

- Ratio of males to females in the brood tanks is typically 1:1 (2:1 is also an option)
- Female pompano target size 3-6 pound range
- Male pompano target size 1-3 pound range
- Our maturation tanks are approximately 1000 gallons stocked with 24 fish (12 males, 12 females)

Feeding

- Pompano have a high energy requirement
- Fish are fed 3-5% of the total biomass in the tank per day
- Feeding frequency is twice a day, morning and late afternoon feedings
- Maturation diet is an enriched powder mix (e.g., Florida Aqua Farms- Gelly Belly Food Mix)

Conditioning

- This allows the operator to manage broodstock maturation through photo/thermal manipulation
- Temp/light cycles are changed every week
- It is important that the cycle not be interfered with once started
- If a disease event occurs, hold the fish at the current stage and do not advance any further
- Once they are healthy and eating well, cycle will commence

Pompano Conditioning Cycle

Week	Light:Dark	Temperature (C)	Comments:
1	12.5:11.5	25	
2	13:11	26	
3	13.5: 10.5	27	
4	14:10	28	
5	14:10	28	
6	13.5:10.5	27	
7	13:10	26	
8	12.5:11.5	25	
9	12:12	24	
10	11.5:12.5	23	
11	11:13	22	
12	10.5:13.5	21	
13	10:14	20	
14	10:14	19	
15	9.5:14.5	18	
16	9:15	18	
17	9:15	18	
18	9:15	18	
19	9:15	18	
20	9.5:14.5	18	
21	10:14	19	
22	10:14	20	
23	10.5:13.5	21	
24	11:13	22	
25	11.5:12.5	23	
26	12:12	24	Hold for Spawning (up to 6 months)

Spawning/Hormone Manipulation

- Pompano are spring spawners with an ideal spawning temp of 24°C and 12 hr light
- Natural spawning of pompano in captivity is unreliable
- Hormone manipulation is necessary for consistent and controlled spawning

Spawning/Hormone Manipulation

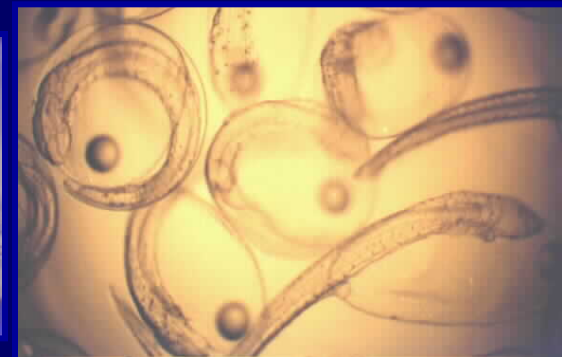
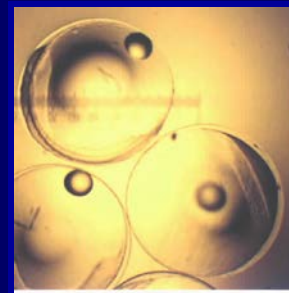
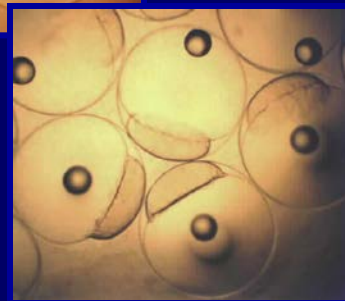
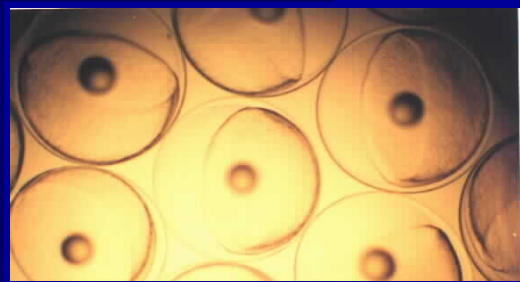
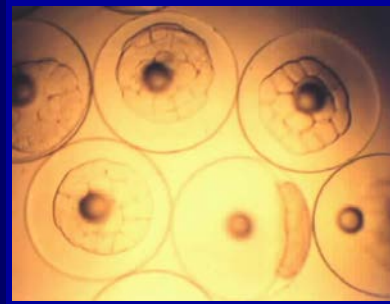
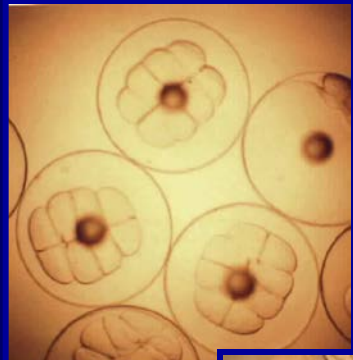
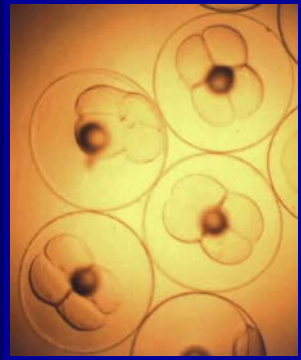
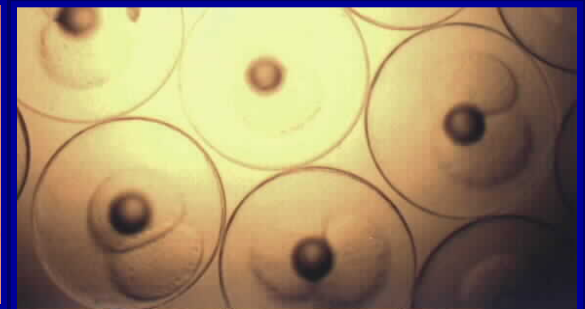
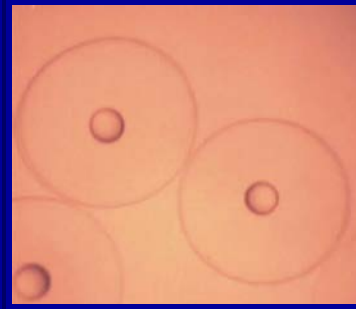
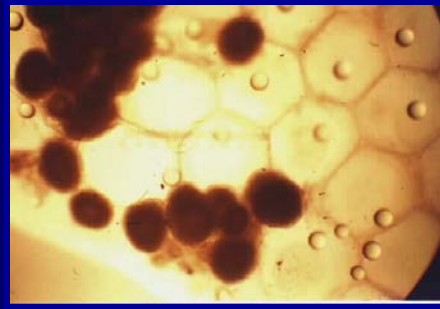
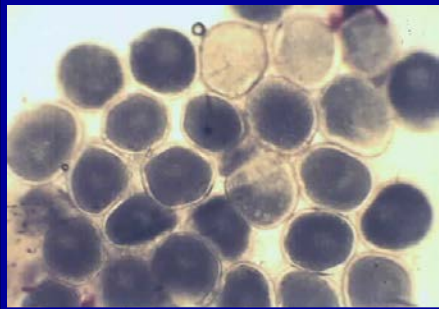
- Once fish have reached a state of gonadal maturation (vitellogenesis), hormones can be administered to achieve an egg release
- Operator can only get the desired spawn when the oocytes (pre-spawned eggs) reach a certain size
- For pompano that egg diameter is greater than 0.5 mm
- At time of release egg diameter will be approximately 1.0 mm

Spawning/Hormone Manipulation

- Salmon Gonadotropin releasing hormone analogue (sGnRHa) – 75 μg dose
- This is a slow release implant which gives the fish an initial burst of gonadotropin followed by several days of lower levels
- Pompano is a high energy fish, however, and will usually yield one viable spawn two days (36hrs) after injection of implant
- Any other residual spawns are typically not viable



OOCYTE, EGG AND EMBRYONIC DEVELOPMENT



Larviculture-Hatching

- Unfertilized eggs can contribute to the fouling of a hatching container
- This can be reduced by ensuring proper fertilization
- Removal of unfertilized eggs will reduce potential for fouling



Larviculture-Hatching

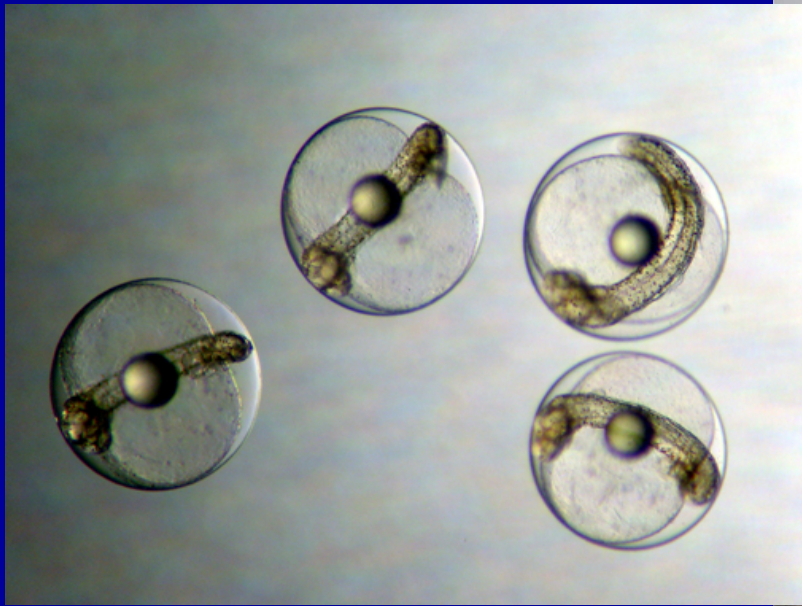
- Disinfectants for keeping bacteria and fungus off eggs:

Betadine, methylene blue, formalin, nitrofurazone, malachite green, perioxide

- With proper water quality, chemicals are rarely needed

Larviculture-Counting of Eggs

- An egg count should be approximated prior to stocking to determine hatch rate and to ensure proper stocking density
- Obviously you are not going to hand count each egg
- Rule of thumb 1 mL of pompano eggs is approximately 1000 eggs (e.g., 40 mL is 40,000 eggs)





Intensive Tank Culture Larval Rearing Systems

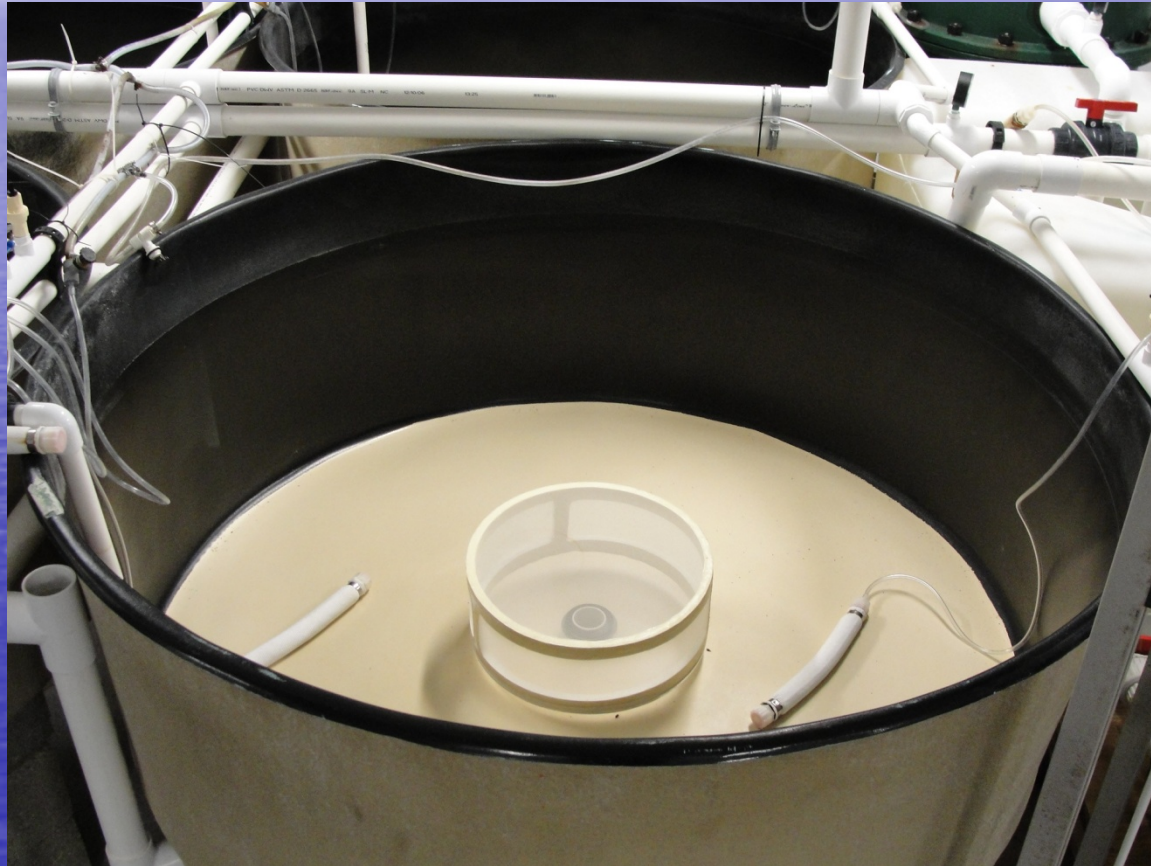
- High stocking densities (50-150 larvae/liter)
- High water exchange rate (minimum 100% turnover rate daily) *or intensive recirculating systems*
- Live microalgae/microalgae paste (*Nannochloropsis spp*; “green water”)
- Rotifers (*Brachionus sp.* enriched)
- *Artemia* (Nauplii + Metanauplii enriched)
- Artificial weaning diets (dry feeds)

Larval Rearing Tanks

- Dark-walled tanks preferred because larvae can see prey better against a dark background.
- Tanks should allow easy viewing, feeding, treatment, and harvesting
- Tank volume: 800 Liters
- Larval exclusion screens of different mesh sizes is required to retain larvae during water exchange (e.g., 150, 350 and 500 micron screens)



Larviculture Tank



Larval Rearing Systems

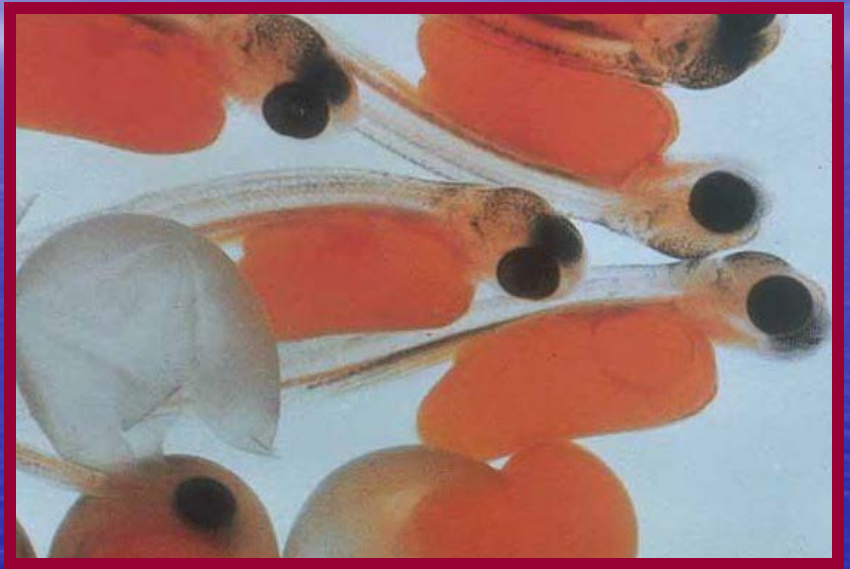
- Tanks should be supplied with air/LOX and water inlets so that individual tanks can be isolated if chemical treatments are necessary
- Recirculating System Components:
 - Biofilter
 - Particulate filtration to 5 microns or less
 - UV sterilization
 - Temperature control (heat pump)





Yolk-sac larvae

- Eggs are stocked into larval tanks
- Eggs hatch the following day (day 0)
- Larvae live on yolk sac for 2-3 days after hatching





LIVE FEEDS (Microalgae, Rotifers, Artemia, Copepods, Plankton) Protocols (maintenance/production/enrichment/disinfection)



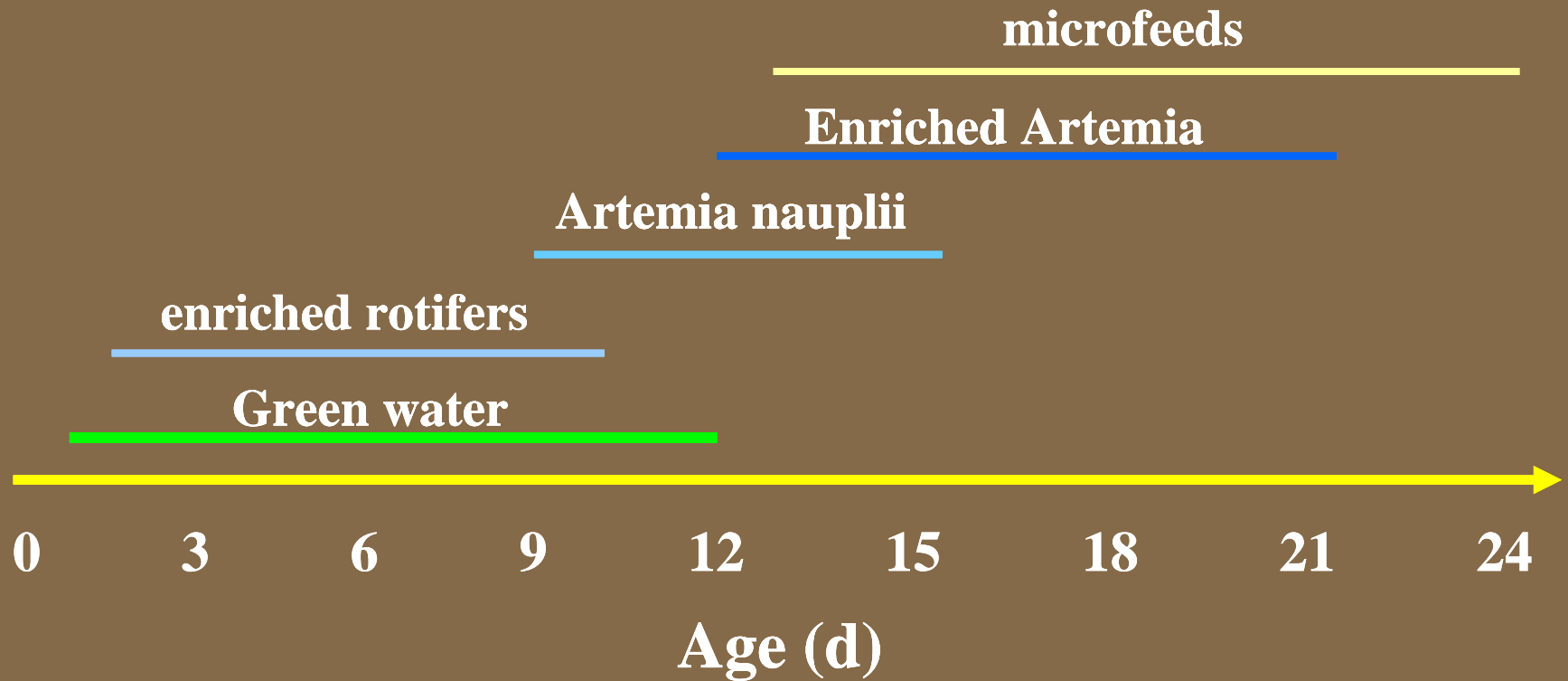
Phytoplankton:
C-Isochrysis sp
Nannochloropsis sp
Diatoms



L, SS Rotifer: *Brachionus spp.*

Brine shrimp: *Artemia salina*

Larval Feeding Protocol for Florida Pompano



Feed Protocol

- Day 0- Eggs hatch
- Day 2- Green water and 1st feeding-enriched rotifers (3 rotifers/mL up to 6 rotifers/mL)
- Day 9- Start introducing newly-hatched artemia (.25-1 artemia/mL up to 4-5 artemia/mL)
- Day 12- Start introducing enriched artemia (2 artemia up to 4-5 artemia /mL)
- Day 14- Start introducing dry feed along with newly hatched and enriched artemia
- Day 22- Larvae should be weened onto dry feed

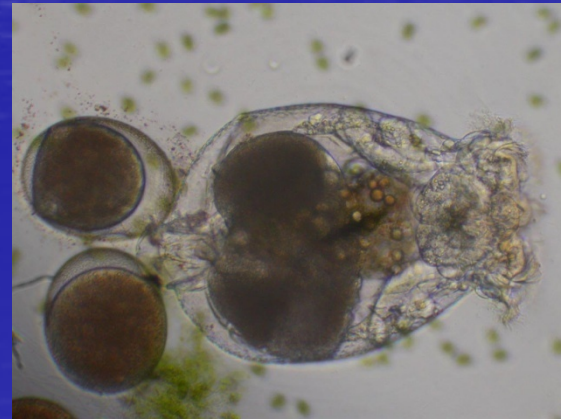
Critical Periods During Larval Development

- First Feeding
- Swim Bladder Inflation
- Feed transition
- Metamorphosis
- Weaning

First Feeding



- First feeding is a critical event for larvae
- Upon opening their mouth, the gut is colonized by bacteria. If these are harmful, the larvae will die.
- Fish larvae are visual predators; therefore must offer live zooplankton as first food



Size of First Prey

- Size of the initial prey item is dependent upon mouth gape
 - Cod - Ciliates, trochophores
 - Grouper, snapper – small copepods
 - Pompano, cobia – rotifers
 - Hybrid striped bass – Artemia

Swim Bladder Inflation

- Fish gulp air at the surface to fill the swim bladder
- Oily films on the water surface can prevent normal swim bladder inflation
- Failure of swim bladder to inflate leads to scoliosis and eventual death



Feed Transition



Metamorphosis

- Metamorphosis is the physical transformation of the fish from its larval form to its juvenile form
- Metamorphosis can be negatively impacted by:
 - Dietary deficiencies
 - Inappropriate temperature regimes (29°C)
 - Poor water quality (D.O.-100%, pH-7.0-7.7, Salinity-35ppt, Alkalinity >100mg/L, TAN<.5mg/L, Nitrite<1.0mg/L)



Weaning

- Weaning is often difficult
- Artificial feeds need to be introduced early, even before fry begin feeding on them so that they are "part of the environment"
- Weaning failure often leads to cannibalism

Bottom line



Vendor List

Vendor	Item
INVE	Artemia Cysts, Sparkle (rotifer supplement), A1 DHA Selco (artemia enrichment), Sanocare Hatch Controller (artemia)
Vero Chemical/Pentair Aquatic Ecosystems	liquid chlorine, isopropyl alcohol, sodium thiosulfate/ Instant Ocean, Defoamer
Reed Mariculture	Algae Paste (rotifer feed/green water), Otohime dry feed (larval feed)
Western Chemical/Syndel	Ori-Green (rotifer enrichment)/ RAL Gun (Hormone injector), Oviplant (hormone)
SCI	Catheter tubing (sample fish eggs from females)
Biomark	Pit tags (tag broodfish)
Florida Aquafarms	Gelly Belly maturation diet premix