Chapter 9

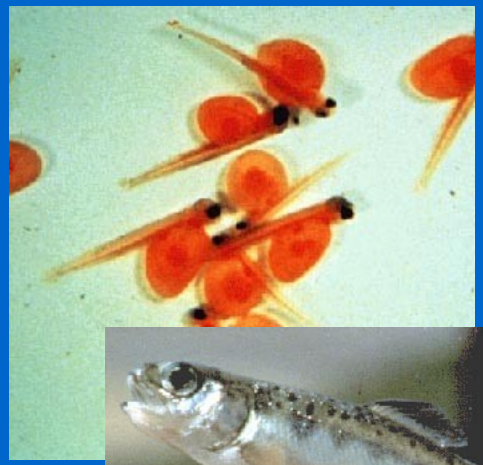
Collection, Preservation, and Identification of Fish Eggs and Larvae

9.1 Introduction
You will learn...

- Methods of collecting, processing and identifying
- Marine and freshwater studies
- Gears used to collect eggs and larvae
- Effects of physicochemical characteristics and larval behavior

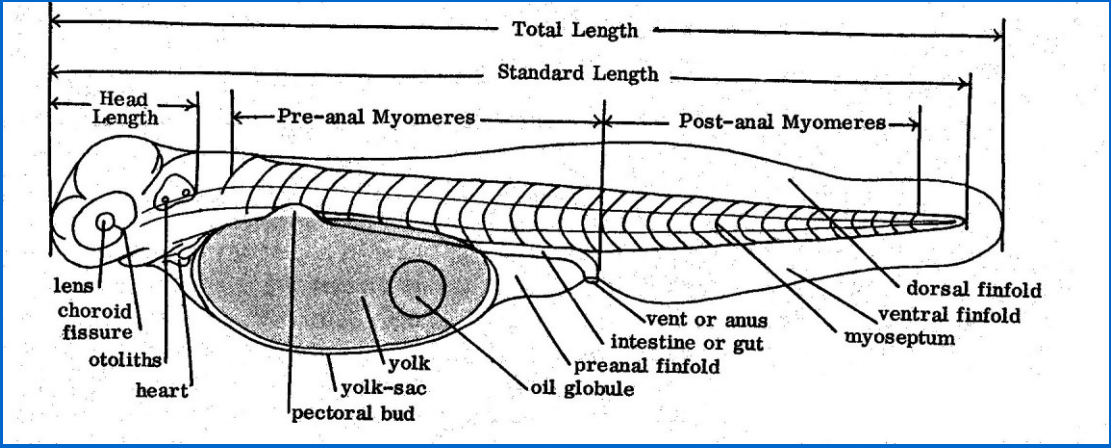
Egg and larval collection important for

- Identification of spawning and nursery areas
- Identification of differences in spawning characteristics
- Ontogenetic changes in movement patterns
- Foraging behavior



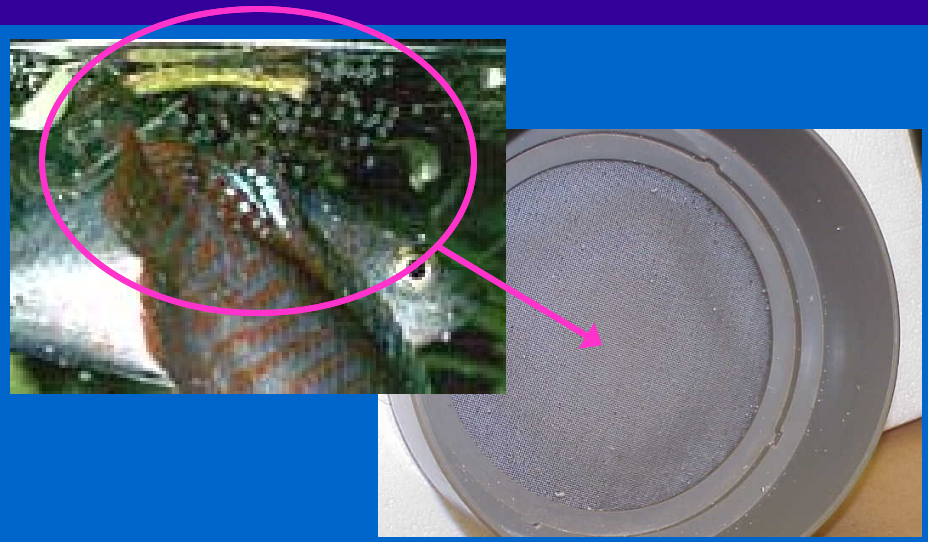
Well designed study requires proper

- Handling
- Preservation
- Identification



9.2 Collection of fish eggs and larvae

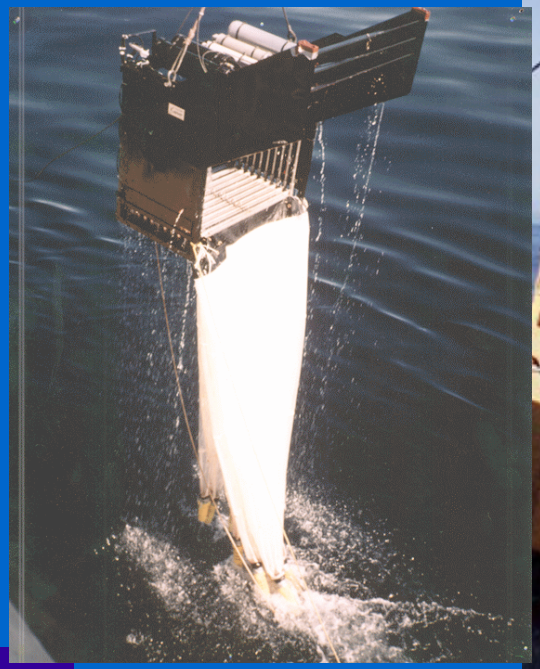
- Pelagic eggs
  - Filtration through fine mesh



- Demersal eggs
  - Use of artificial substrates and traps

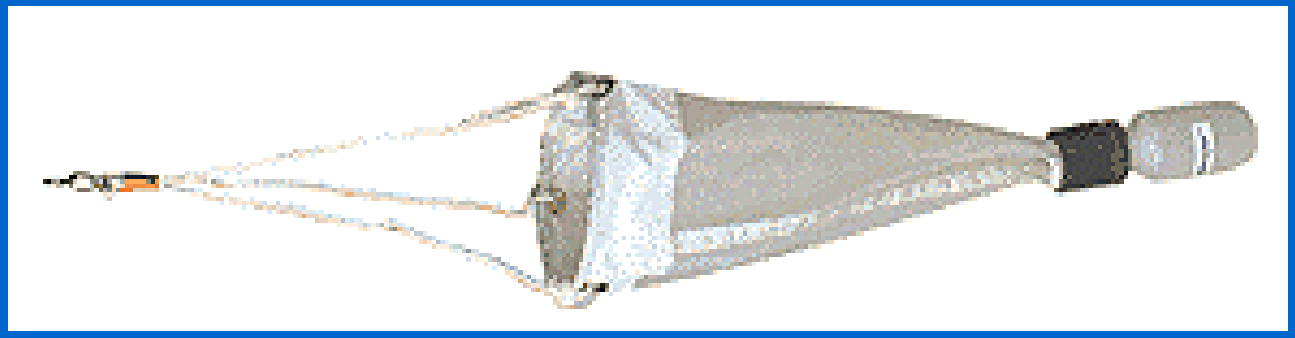
Considerations of gear

- Expense
- Ease of use
- Relative effectiveness
- Sampling bias



Plankton nets

- **Usually:**
  - **Diameter of 0.1m-1m**
  - **Nylon mesh cone or cylinder cone**
  - **Ends in plankton bucket**



Benthic plankton samplers

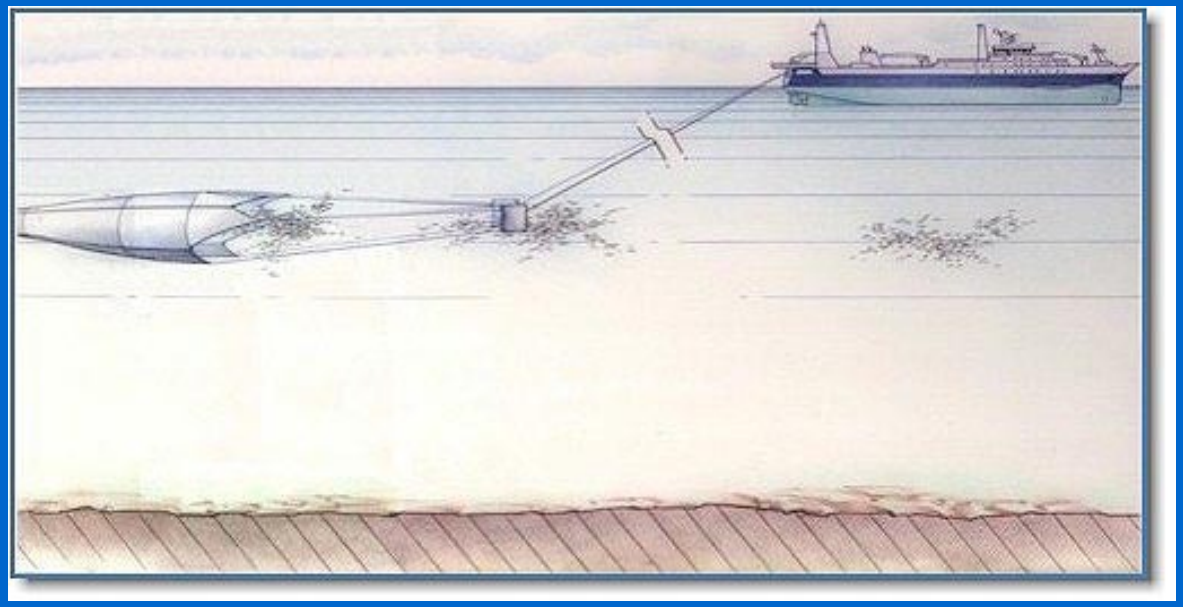
- Sample larvae or eggs on or just above bottom

- Frolander and Pratt-mounted a cylindrical net on a benthic skimmer

Benthic plankton samplers (cont.)

- Dovel-used larger net on benthic sled
- Yocum and Tesar- plankton net on rectangular sled frame

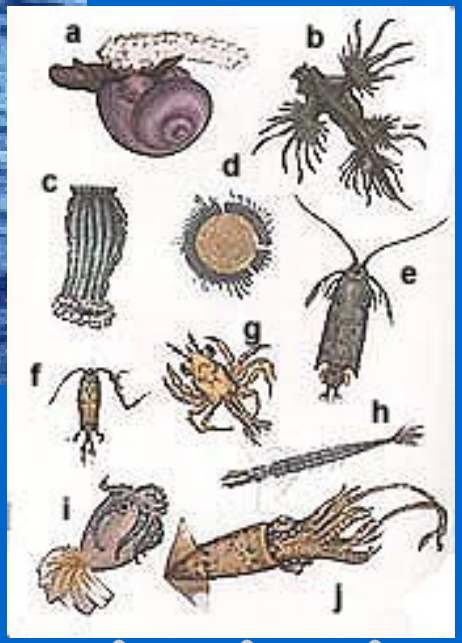
Pelagic Trawls



- Used to sample eggs and larvae in mid-water
- Known as mid-water trawls

Neuston nets

- Towed with the top above water surface
- Samples neustonic organisms

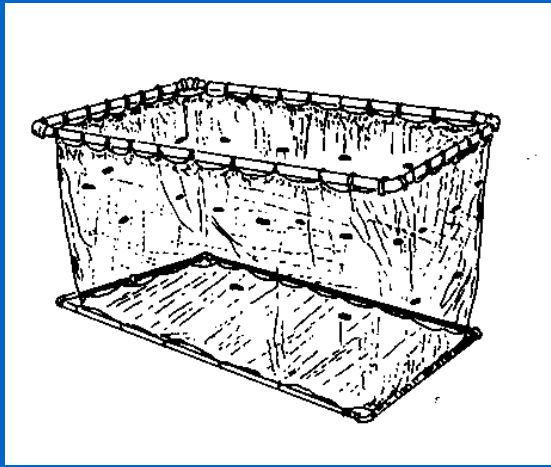


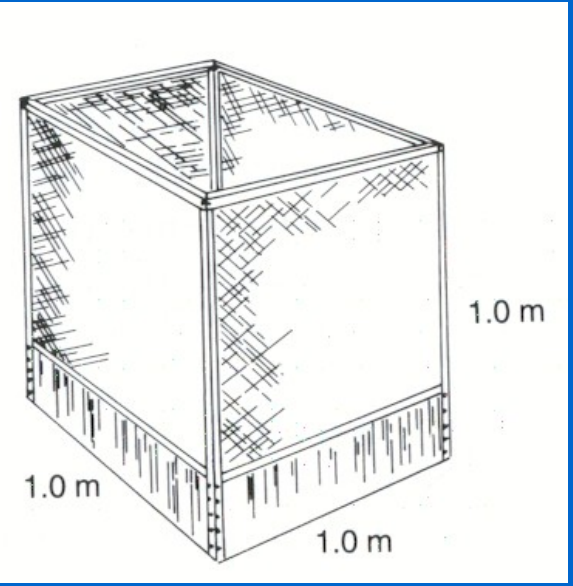
Active Collecting-High Speed Gears

- Collect marine and freshwater ichthyoplankton
- Samplers are typically large

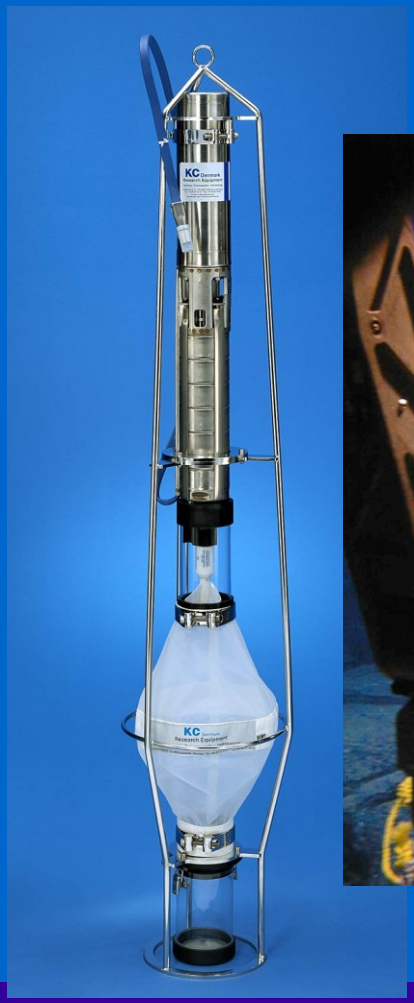
Shallow-Water Nets

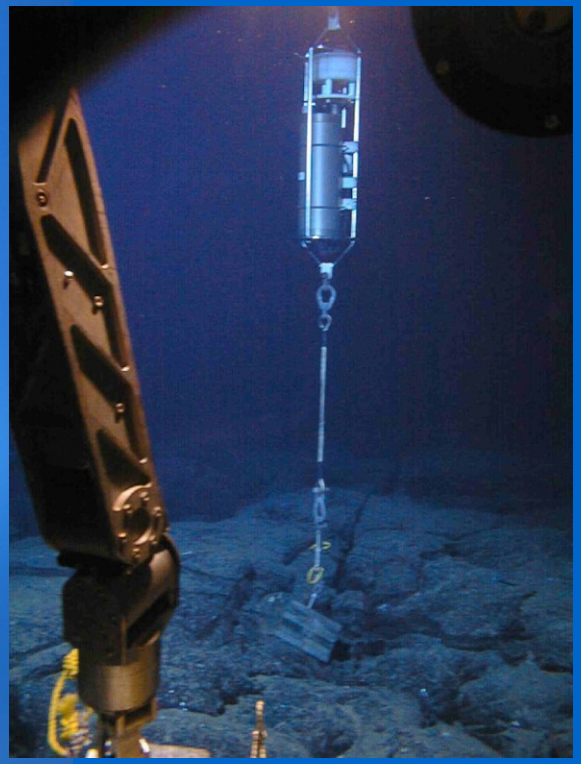
- Shallow areas
- Structurally complex areas





Pumps





- Centrifugal pumps used to collect demersal eggs and larvae
- Study the spatial distribution of pelagic ichthyoplankton

Pumps...Disadvantages

- Pumping volumes small
- Filters and screens can clog
- Pumping area limited to several centimeters of pump intake
- Most larvae are killed or damaged during sampling

Electrofishing gear

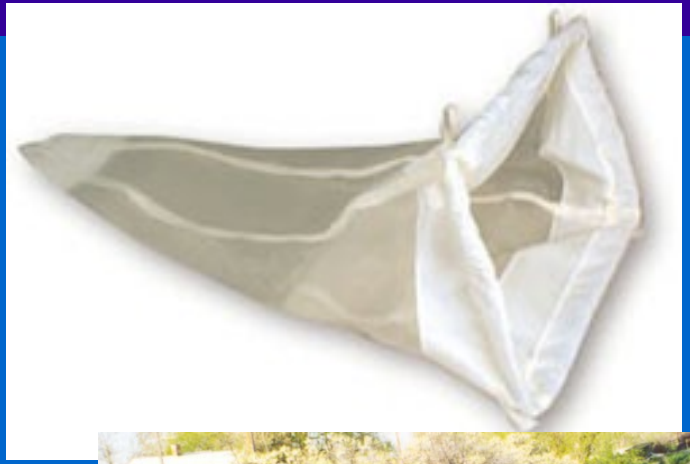
- Not widely used to sample larvae
- Good for shallow or structurally complex areas

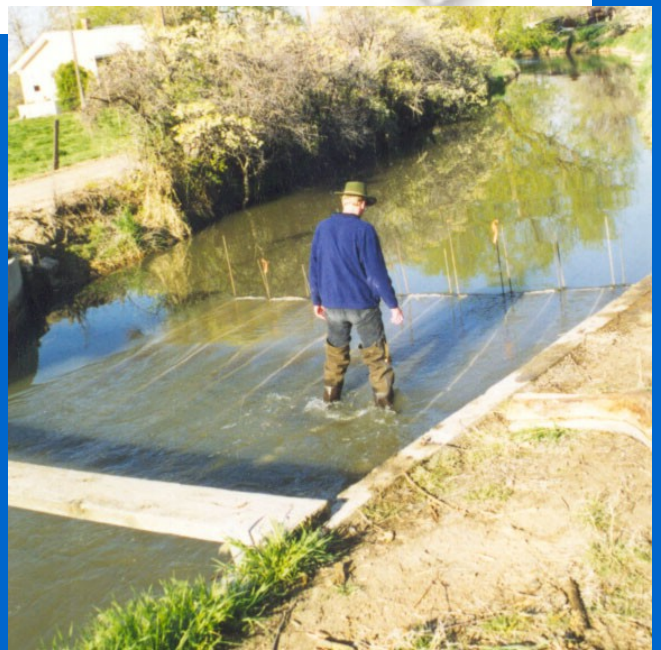
Passive Collecting Gears

- Egg Traps
  - Capture and protect demersal eggs
  - Prove more effective than other methods in number and percentage undamaged

Passive Collecting Gears (cont.)

- Drift Samplers
  - Drifting eggs and larvae collected with stationary plankton nets
  - Both at bottom and top of water column
- Mesh size depends on
  - Size of target organisms
  - Mesh clogging tendencies



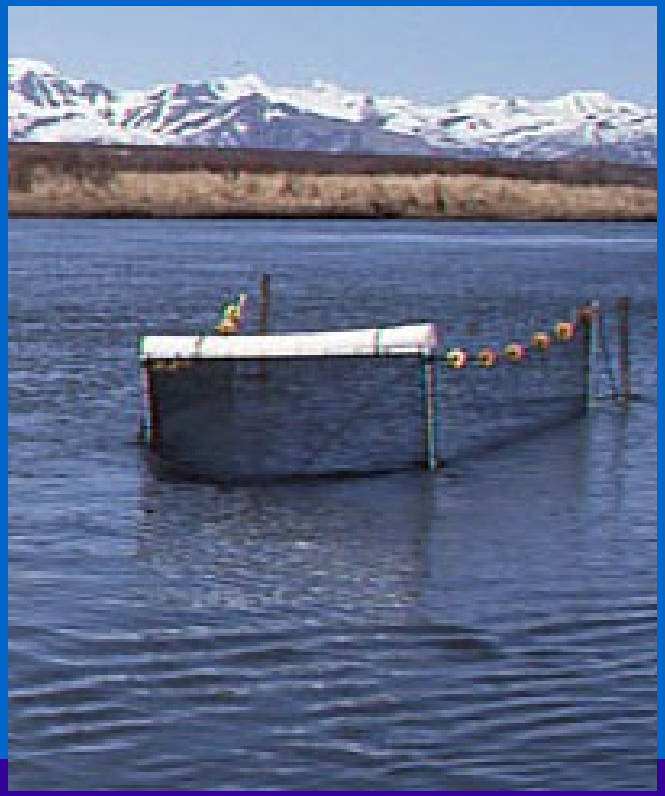


Emergence traps

- Sample the larvae as they leave the nest (emerge)

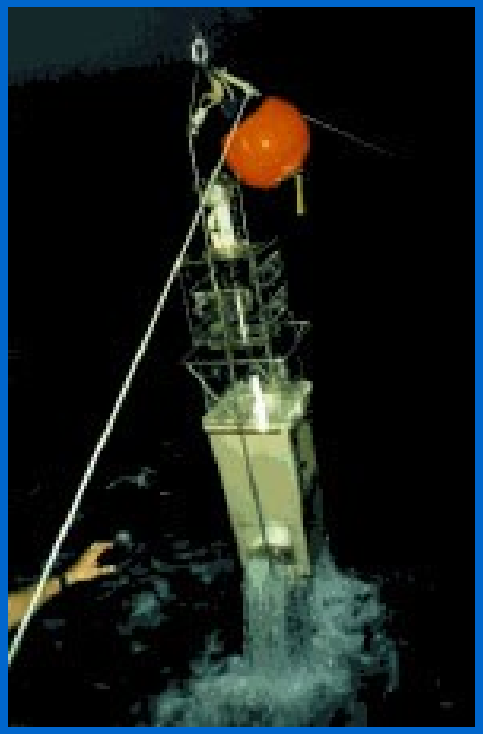
Activity Traps

- Free swimming larvae and juveniles in littoral habitats



Light traps

- Larvae that are positively phototactic
- Used at night (nocturnal)



Sampling Considerations

- Formulation of specific research objectives

How many are there?
Where are they?
When are they?

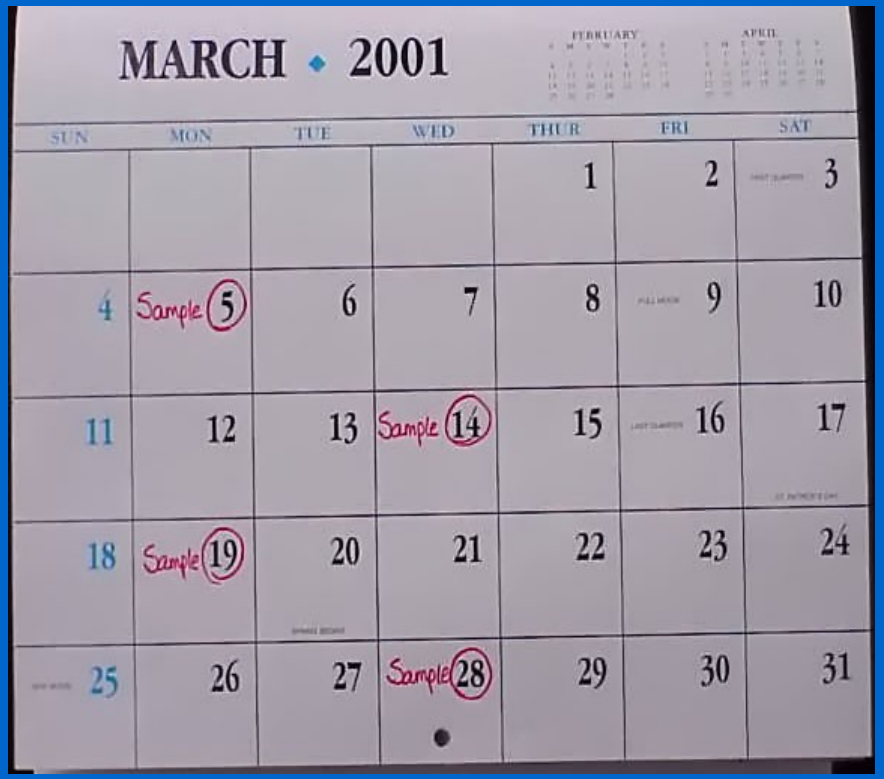
Sampling Considerations (cont.)

- Development of a study design. Affected by:
  - Budget
  - Personnel
  - Equipment
  - Time limitations
  - Biological, ecological physiological and statistical factors

Sampling Considerations (cont.)

- Development of collection methods important
  - Knowledge of fish reproductive behavior
  - Larval behavior and ecology

Sampling Considerations (cont.)

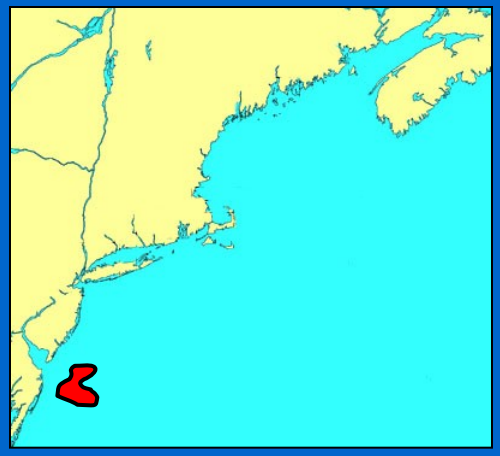


- Gear types
- Sampling periodicity
- Sampling habitat

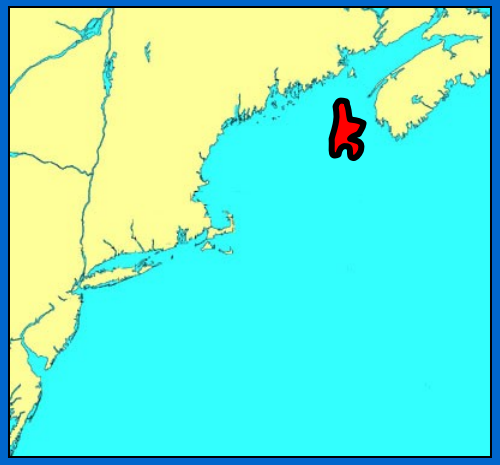
Spatial and Temporal Effects on Sampling Design

- Distribution of fish eggs and larvae vary

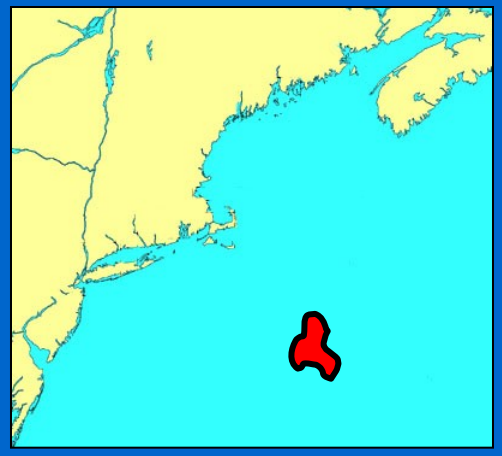
April



May



June



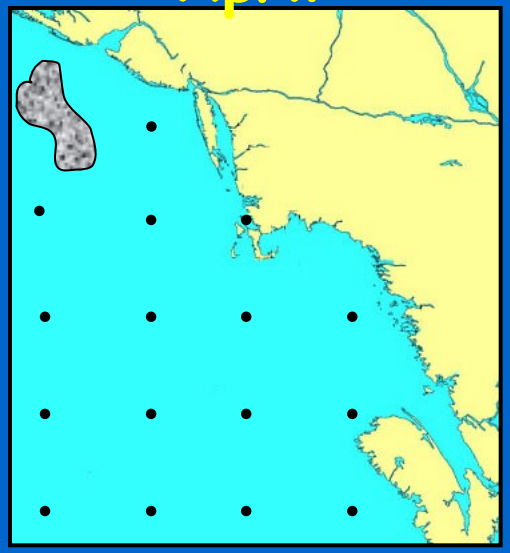
Temporally

- Seasonal variability
- Annual variability
- Temperature
- Physicochemical variables

Spatially

- Must be accounted for in study design

April



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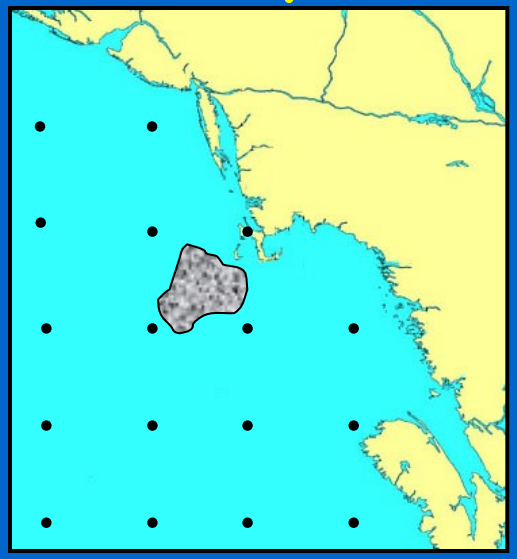
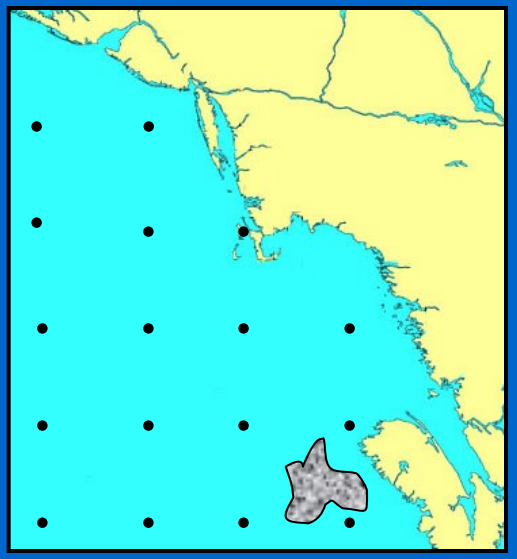
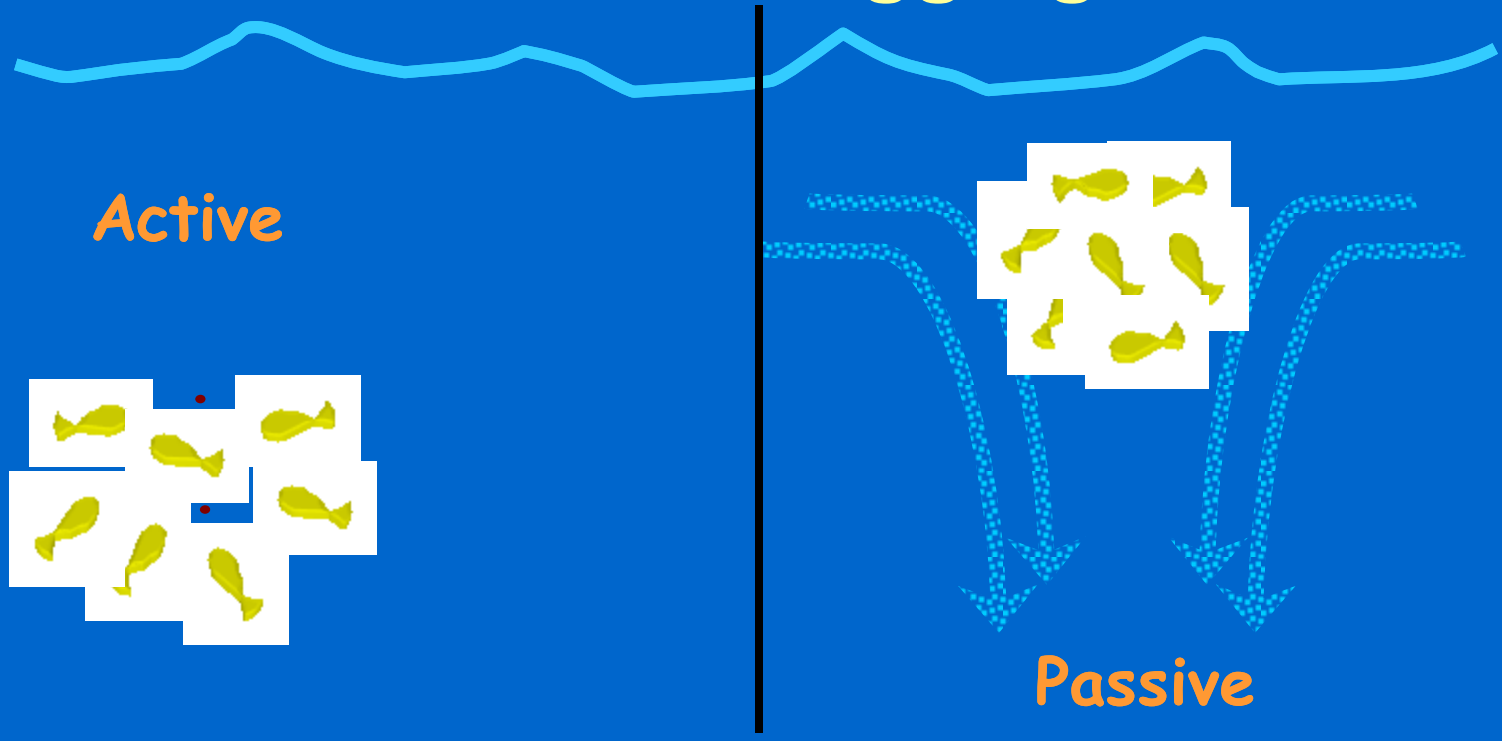


June



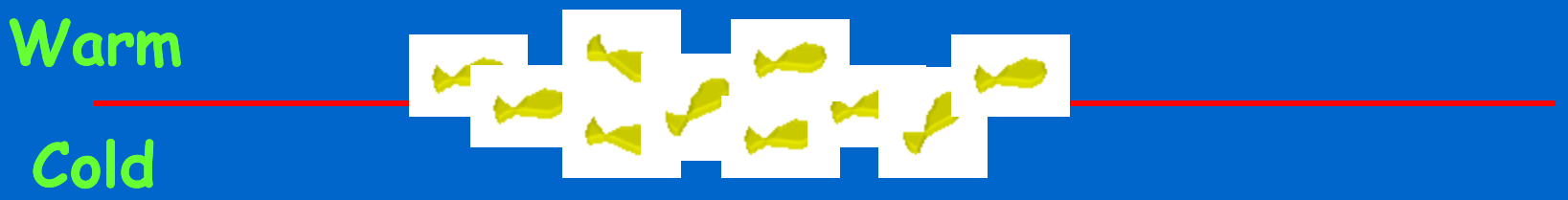
Marine Systems

- Horizontal and vertical patchiness
- Passive and active aggregation



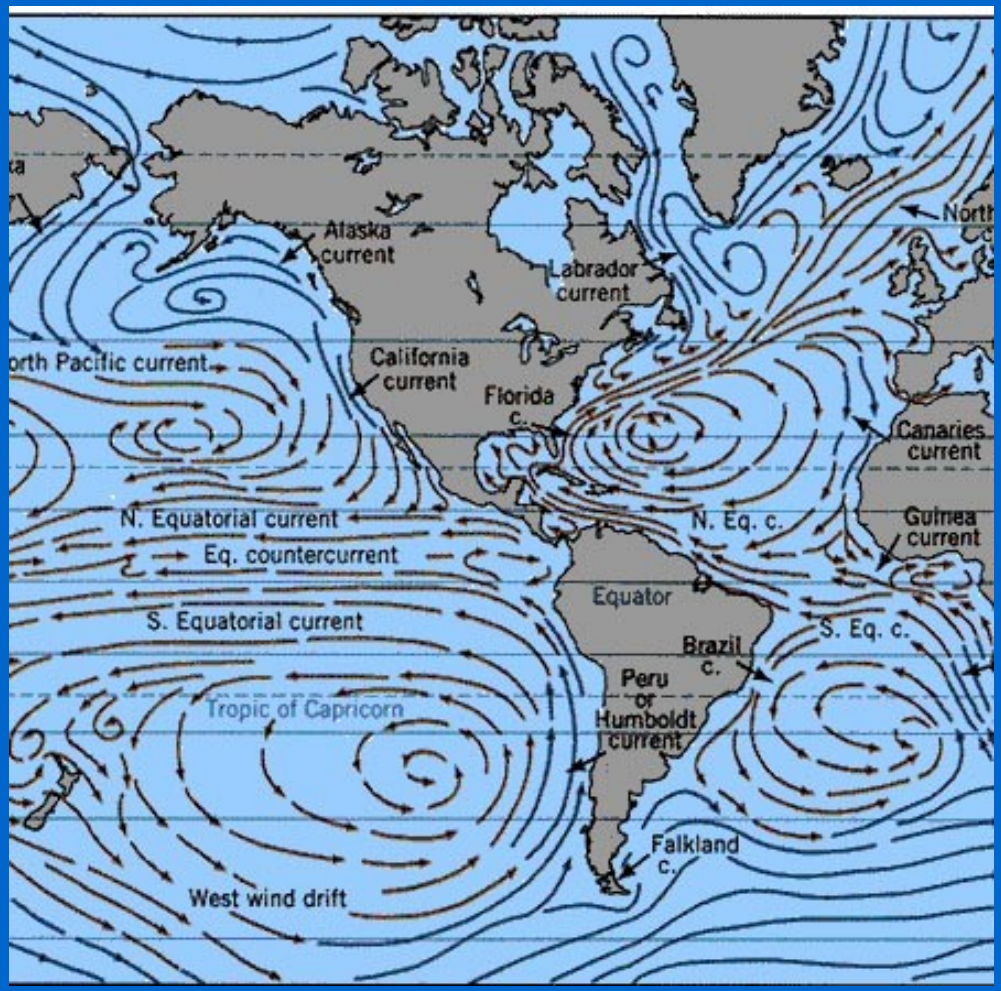
Vertical patterns of distribution depend on

- Egg and larval buoyancy
- Larval behavior
- Temperature patterns



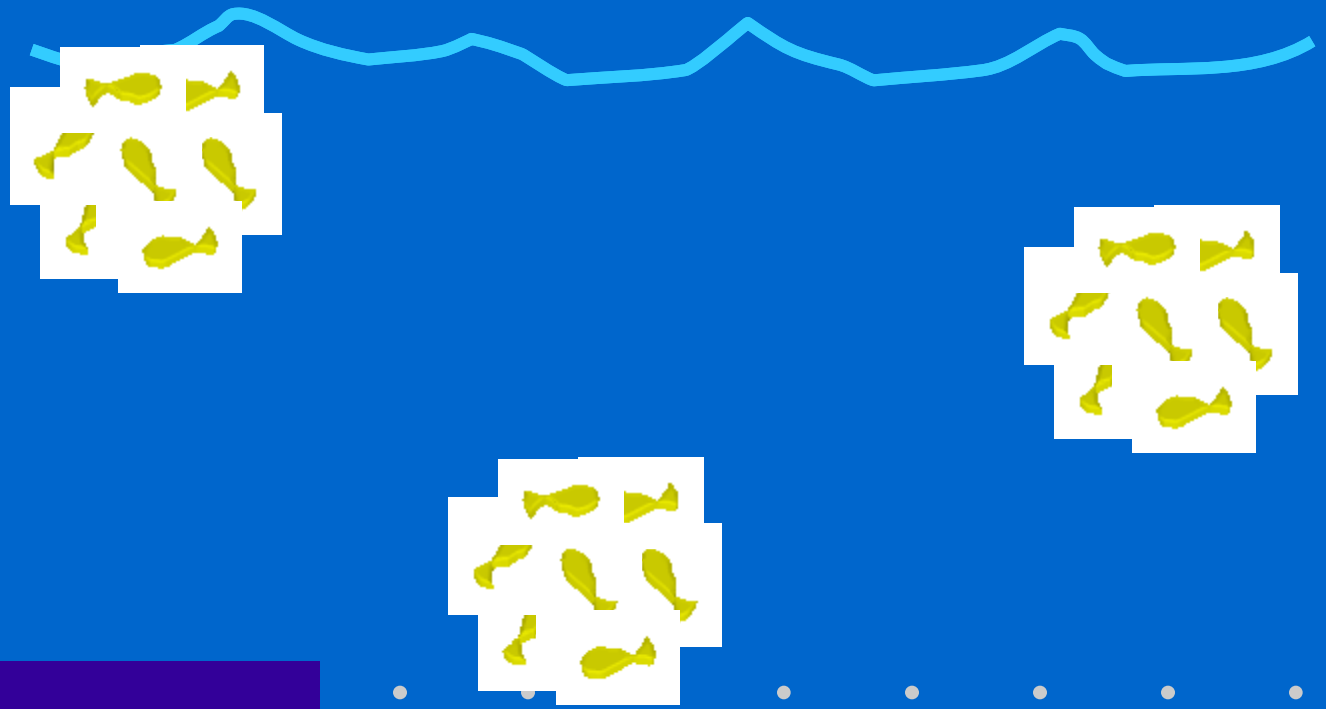
Vertical patterns of distribution also depend on

- Current patterns
- Salinity
- Light
- Distribution and movement of predator and prey



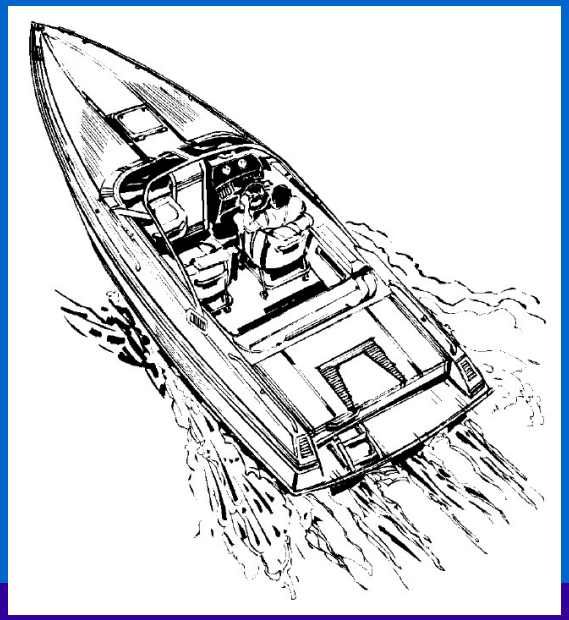
Fish Density/Sample Volume Effects on Sampling Design

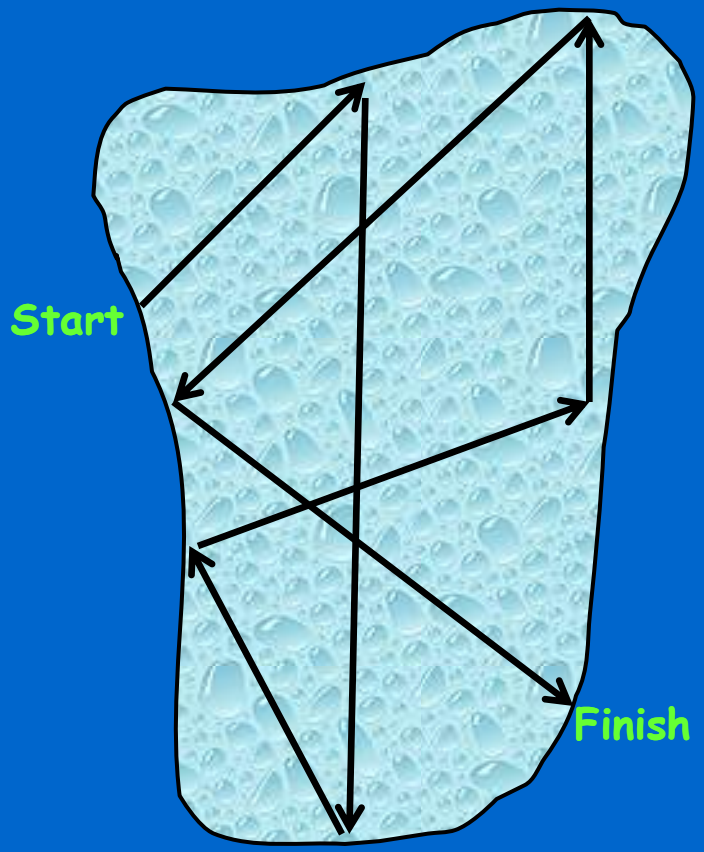
- Consider discontinuities of ichthyoplankton
  - Horizontal
  - Vertical
  - Temporal



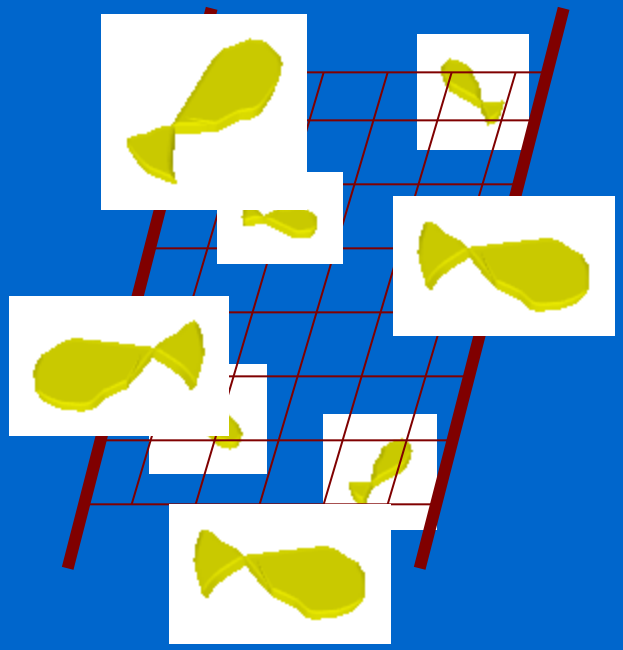
Species and size composition can be affected by

- Volume sampled
- Towing path
- Towing speed





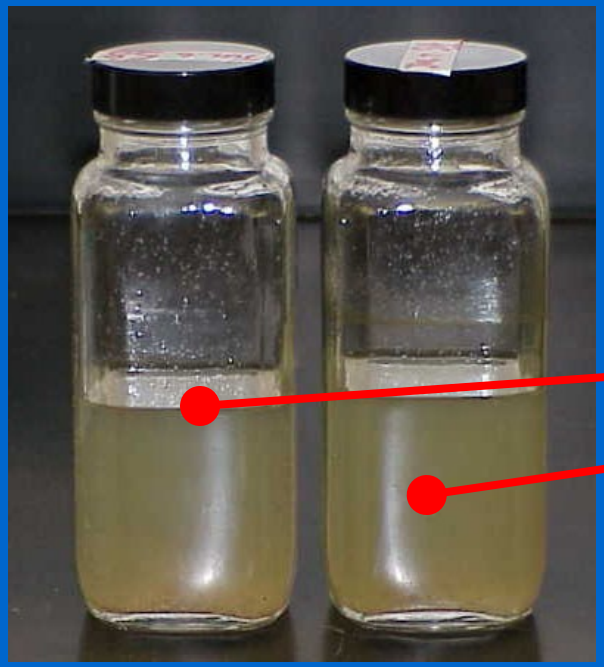
Statistical Considerations



- Biases can occur due to:
  - Extrusion of small larvae through net mesh
  - Net avoidance by larger larvae

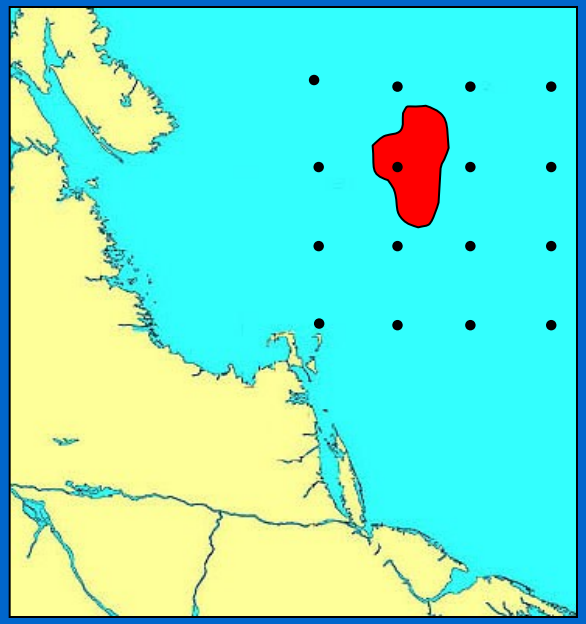
Replication

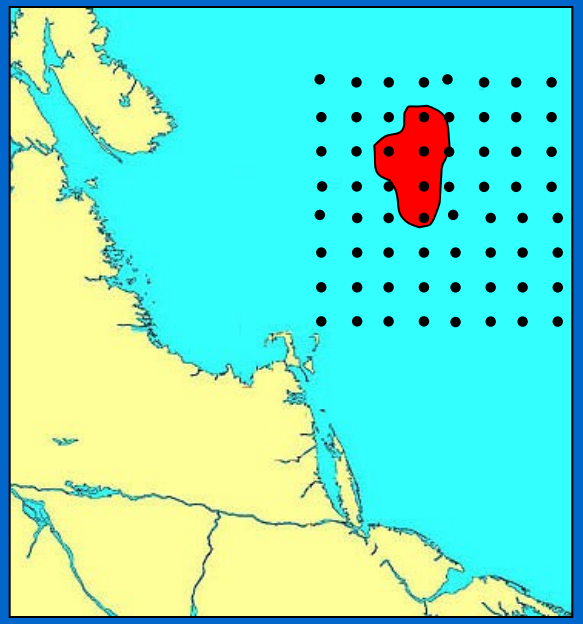
- Allows for estimation of between sample variance



Accuracy

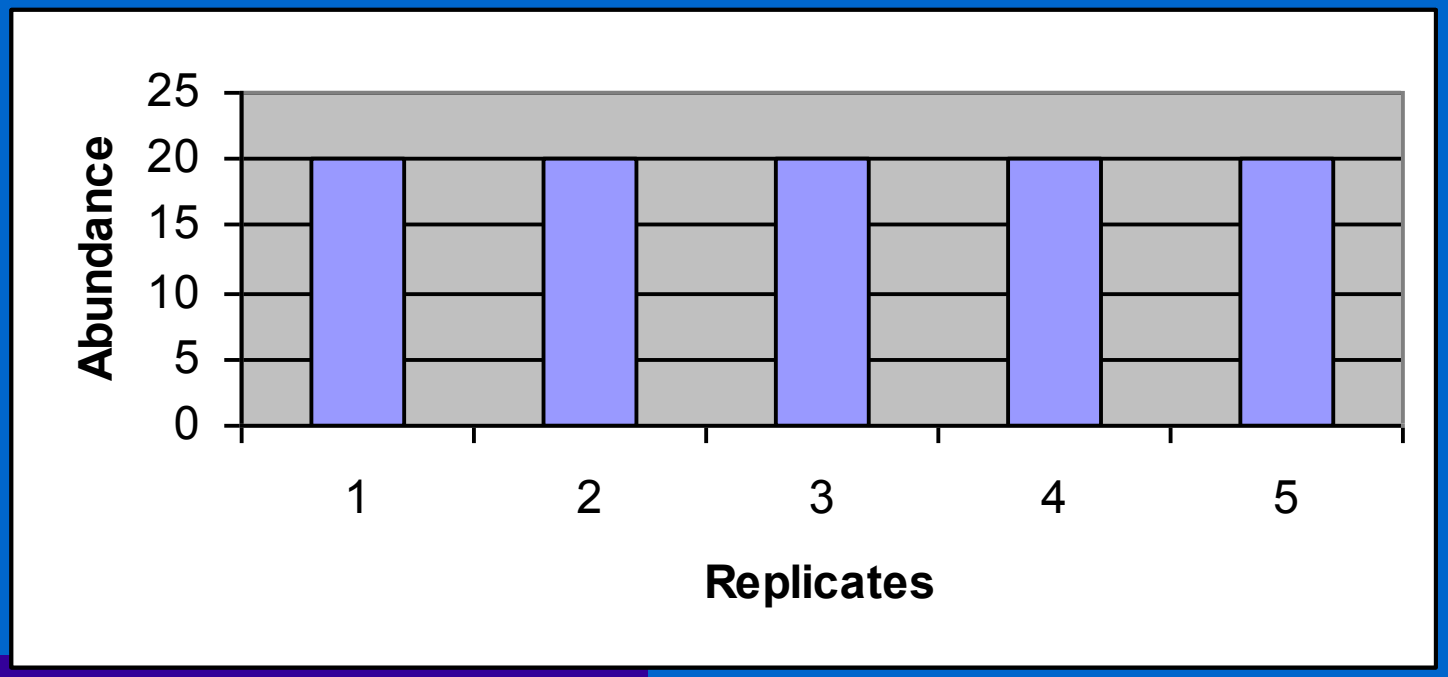
- Depends on ability of sampling design to effectively describe egg and larval characteristics





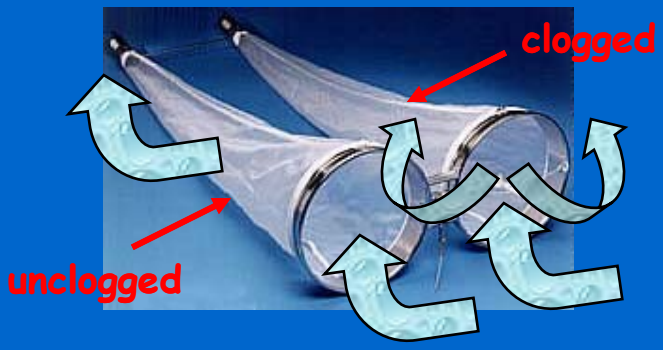
Precision

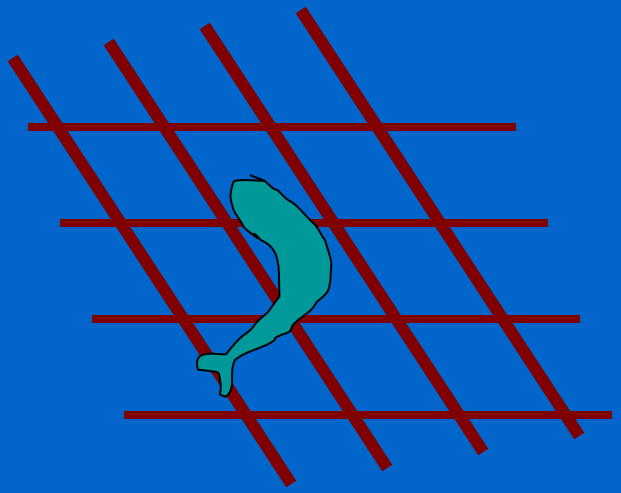
- Strongly affected by ichthyoplankton patchiness and number of samples taken



Effects of Gear Characteristics on Sampling Design

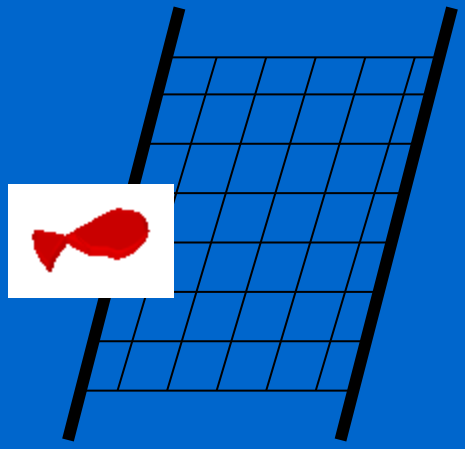
- Clogging of nets
  - Unequal sampling
  - Inaccurate data
- Mesh size
  - Condition of fish
  - Number of fish
  - Species

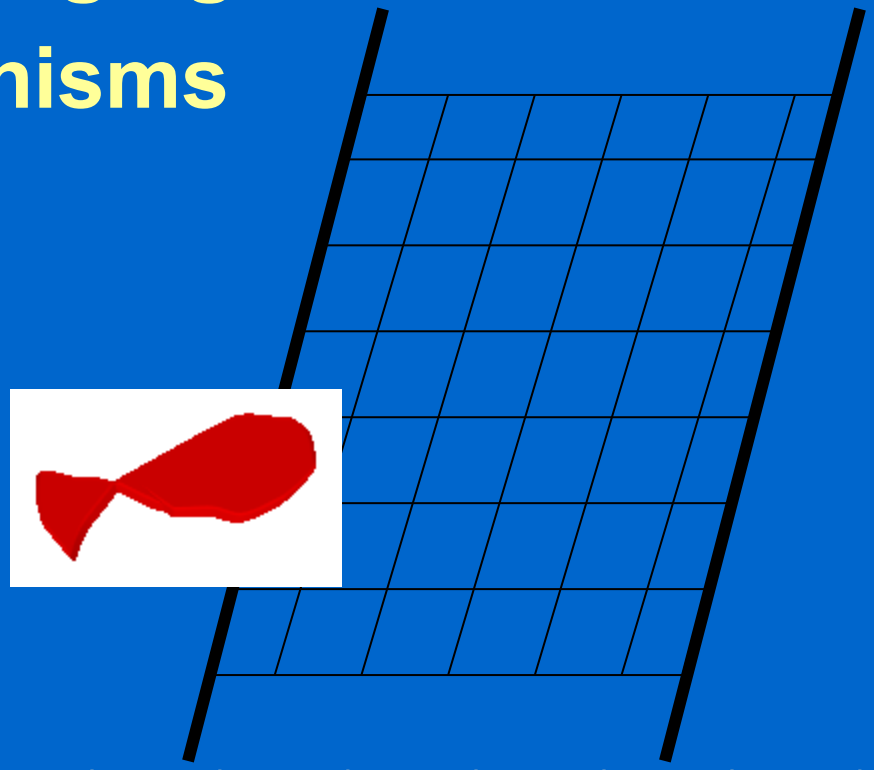




Choice of mesh size depends on

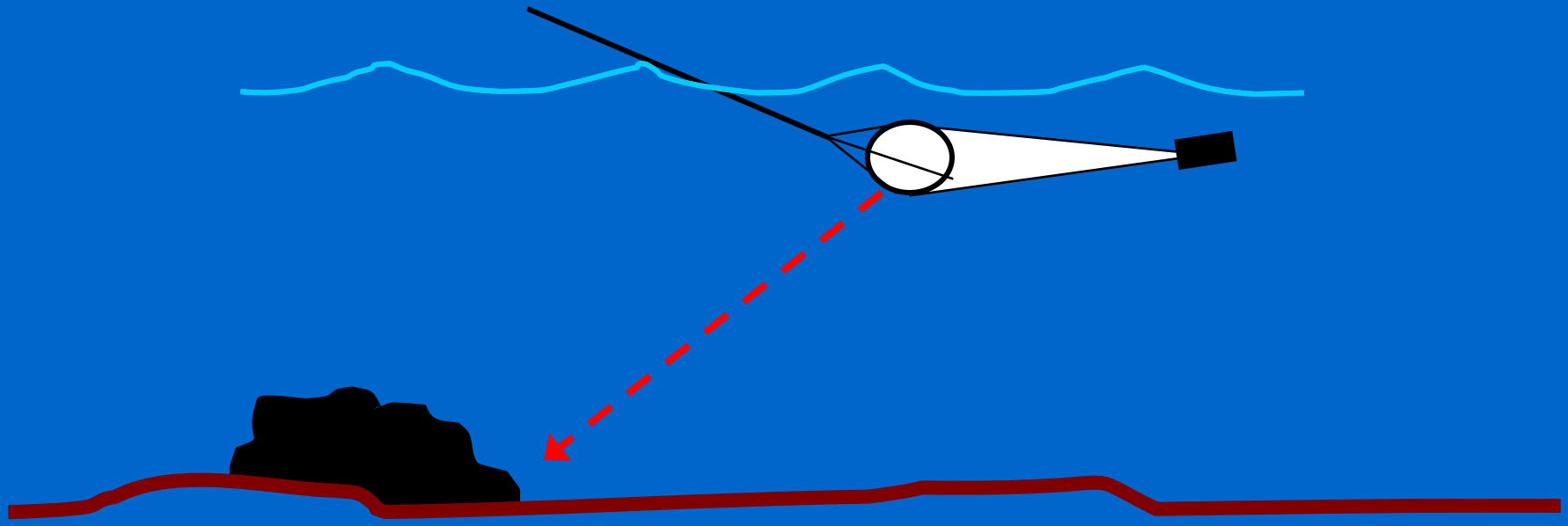
- Gear type
- Water velocity through gear
- Size of target organisms





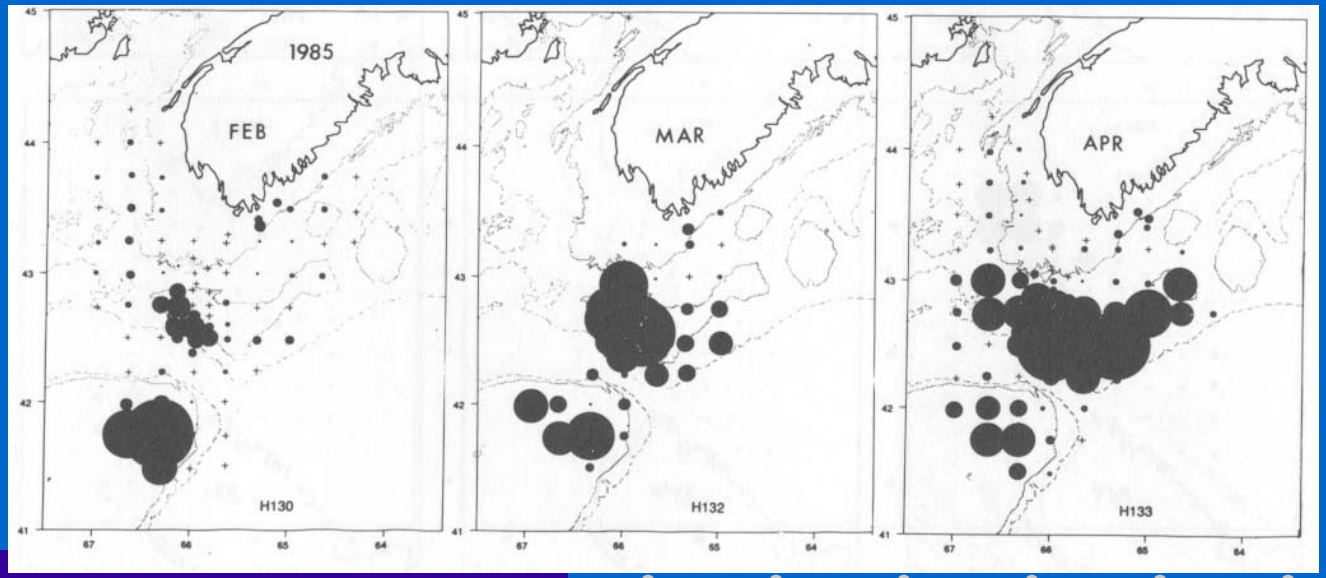
Gear failure can occur due to

- Mechanical problems
- Operator inexperience
- Collision with debris or substrate



Effects of Fish Behavior on Sampling Design

- Important effects on
  - Where
  - When
  - How early life stages are collected



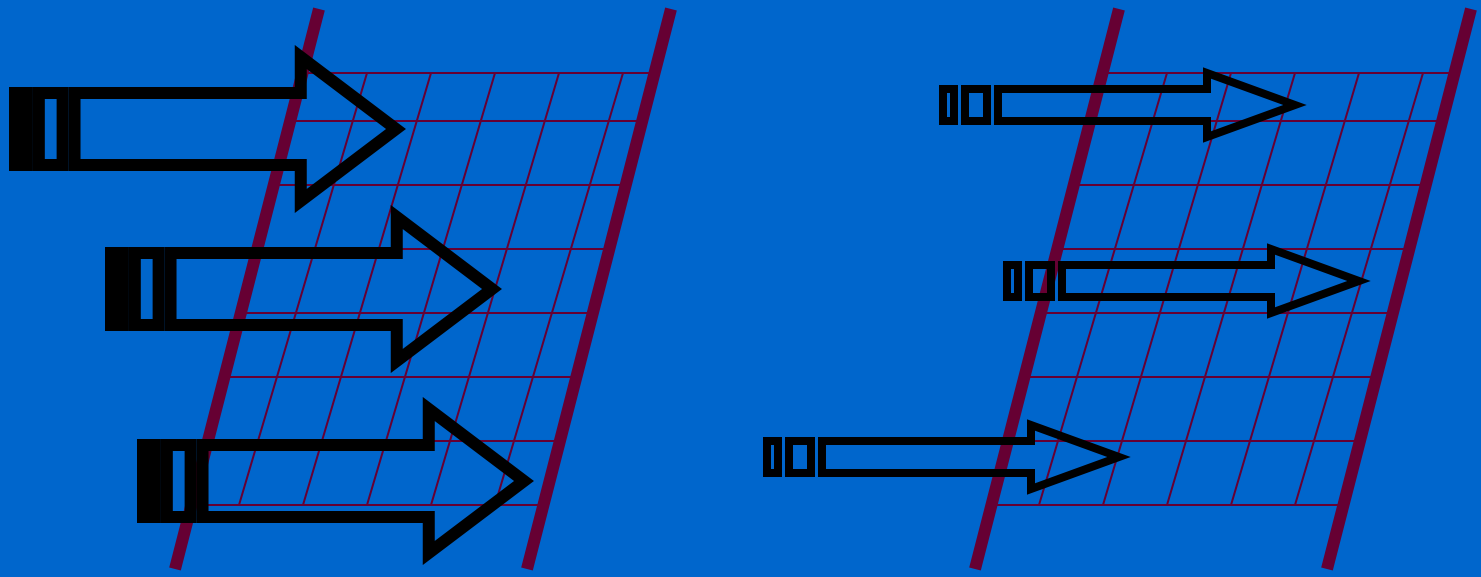
Active avoidance of towed nets and pumps is related to

- Larval size and position relative to net

- Light levels
- Physical characteristics of sampling gear

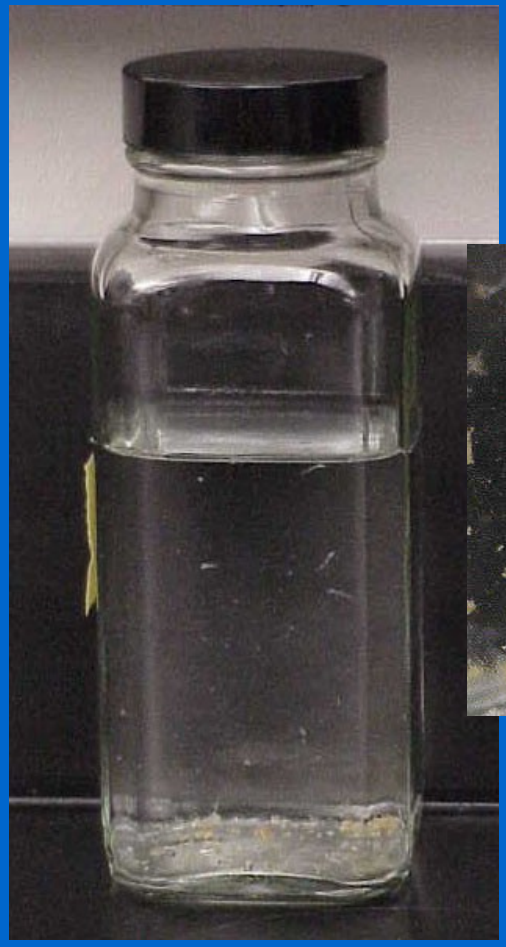
Active avoidance is related to (cont.)

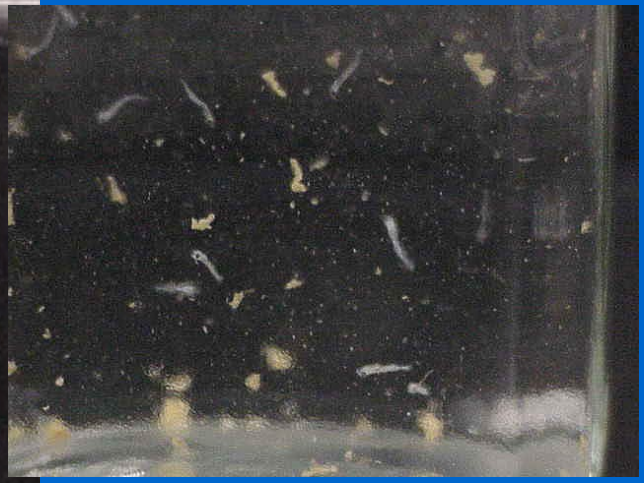
- Velocity of gear or water flow into the gear
- Visual signals
- Hydrostatic pressure waves



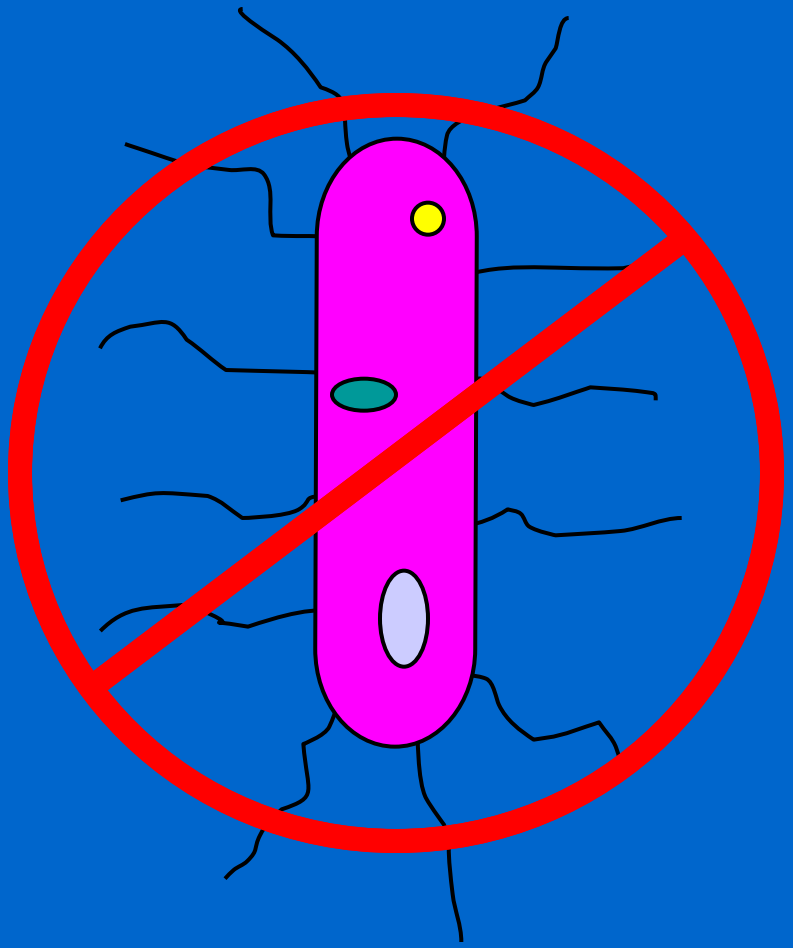
9.4 Sample Preservation

- Important for
  - Taxonomic studies
  - Ecological studies





Fixation method should prevent



- Microbial degradation
- Autolysis
- Cellular damage due to osmotic changes

Degree of degradation depends on

- Developmental stage
- Chemical concentration
- Osmotic strength

High Degradation

Low Degradation

Fixation and Preservation

- All use aldehyde-based solutions (eg. formaldehyde and glutaraldehyde)
  - can be reversed by washing

Formaldehyde preferred

- Less noxious
- Less expensive
- Superior long- term preservation

But...formaldehyde

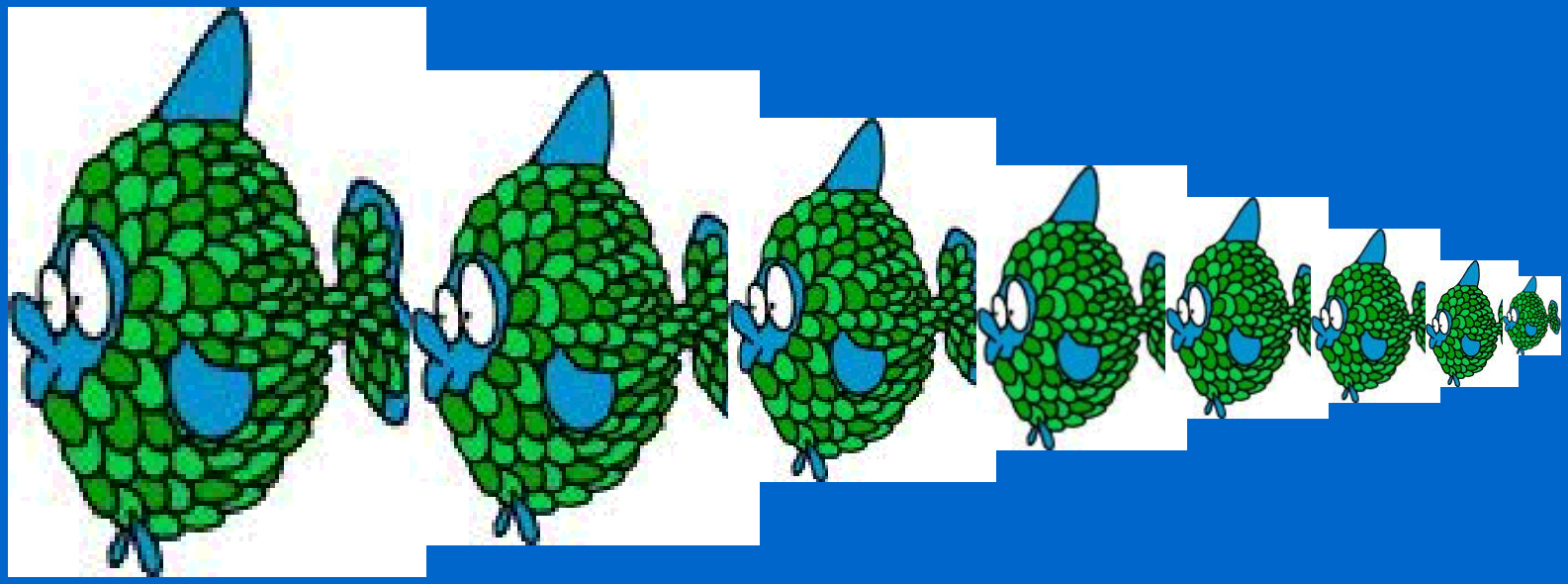
- Is acidic and causes decalcification and demineralization of bone

Formaldehyde can be buffered using

- Sodium borate
- Calcium carbonate
- Sodium phosphate
- Sodium acetate

Alcohol can be used but:

- Cause significant shrinkage and deformation due to dehydration

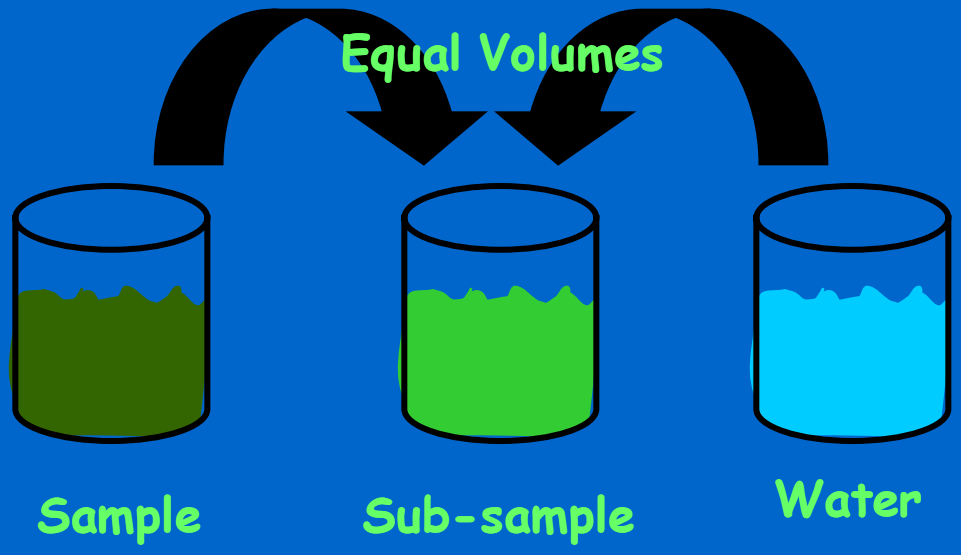


Sample processing

- Immediate processing important
- Returned to the lab for
  - Sorting
  - Enumeration
  - Identification
  - Measurement...etc.

Sub-sampling

- Necessary only if densities of desired organisms is high

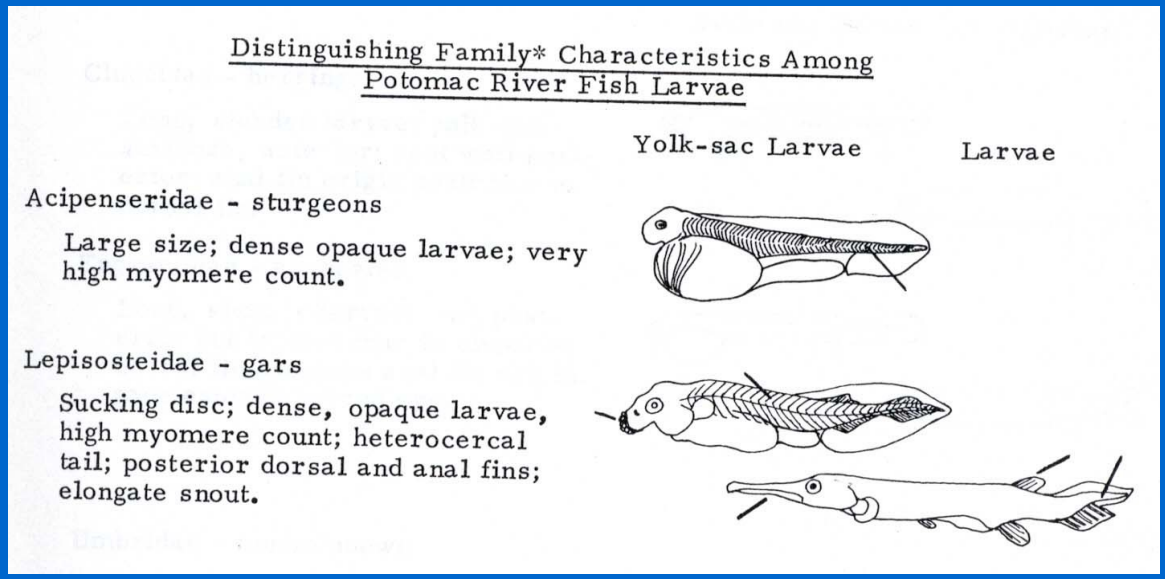


Sorting

- Separate eggs and larvae
- Fixative washed out
- Well ventilated room
- Dye can be used
- Microscope helpful

Terminology and Identification

- Should be done with considerable evidence from
  - Individual and comparative descriptions
  - Regional keys and manuals
  - Reference collections
  - Taxonomic experts



Distinguishing Family* Characteristics Among Potomac River Fish Larvae

Yolk-sac Larvae

Larvae

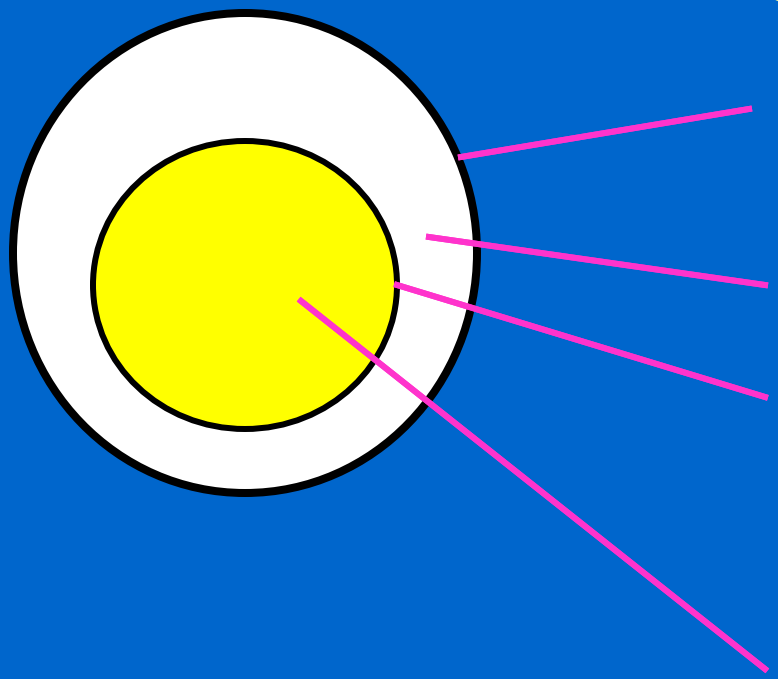
Acipenseridae - sturgeons

Large size; dense opaque larvae; very high myomere count.

Lepisosteidae - gars

Sucking disc; dense, opaque larvae, high myomere count; heterocercal tail; posterior dorsal and anal fins; elongate snout.

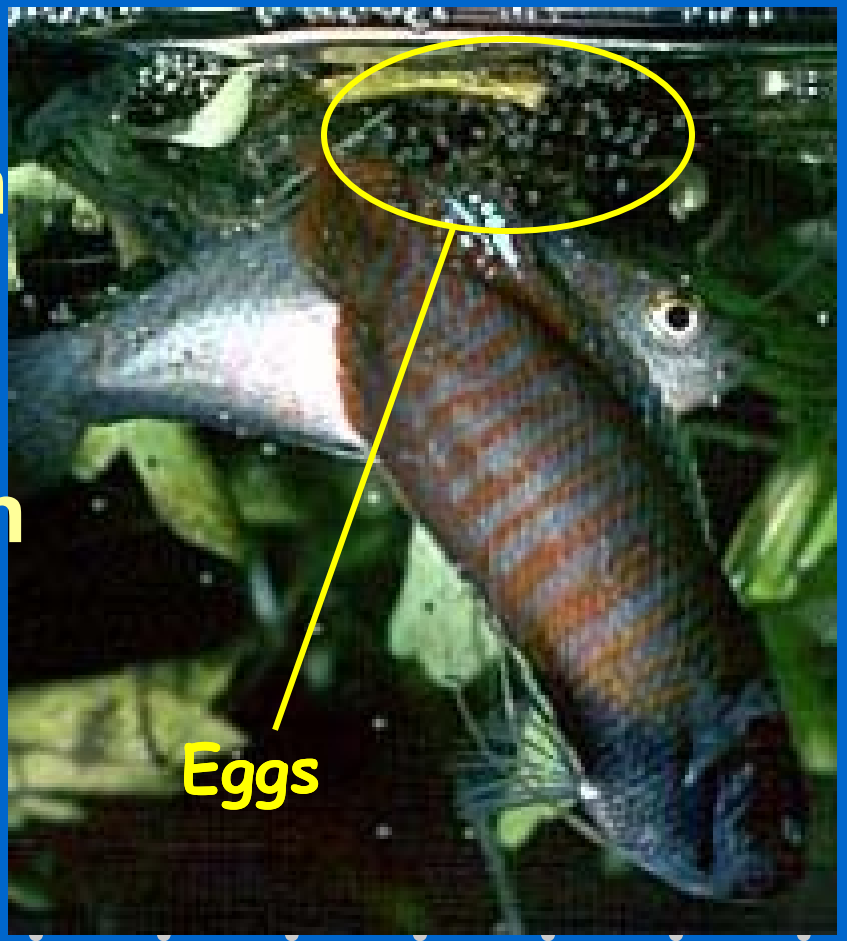
Egg Developmental Stages (ovulation-hatching)



- Egg structure consists of
  - Outer membrane (chorion)
  - Perivitelline space
  - Inner egg membrane (only some fishes)
  - Egg yolk

Most fish oviparous

- Ovulation followed by release of eggs to environment
- Eggs fertilized by sperm from males
- Eggs undergo changes in structure and function
  - Egg activation to prevent polyspermy
  - Chorion hardening

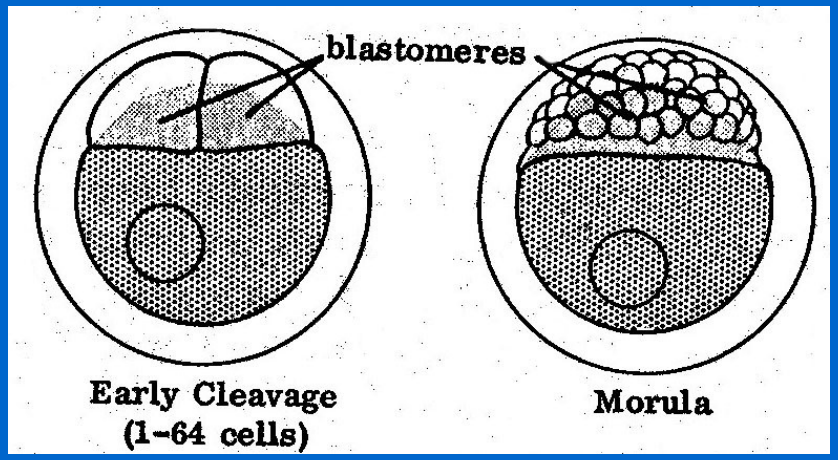


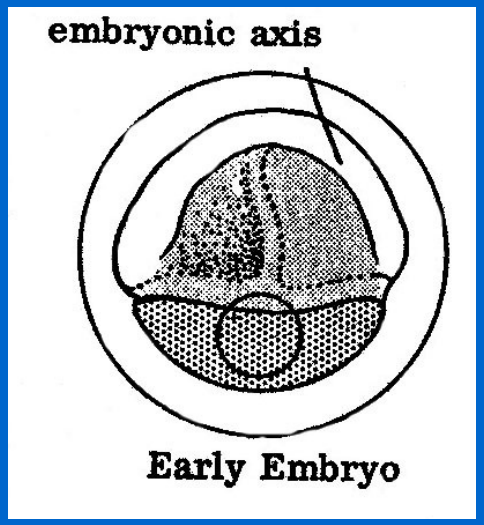
Cell division

- Meroblastic (common)
- Holoblastic
- Intermediate

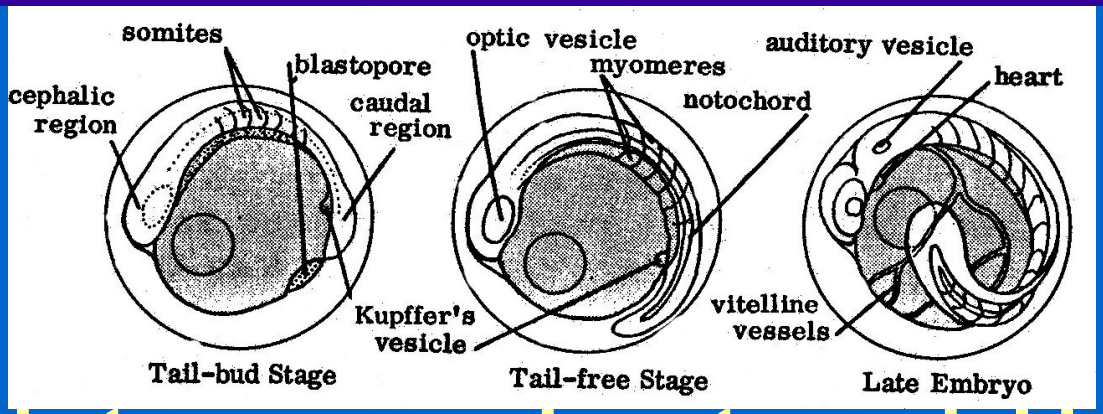
Stages of egg and embryo development

- Early cleavage, 1-64 cells
- Morula, blastomeres that form a cluster of cells
- Ectoderm, mesoderm and endoderm
- Early embryo, formation of the embryonic axis





Stages of egg and embryo development (cont.)



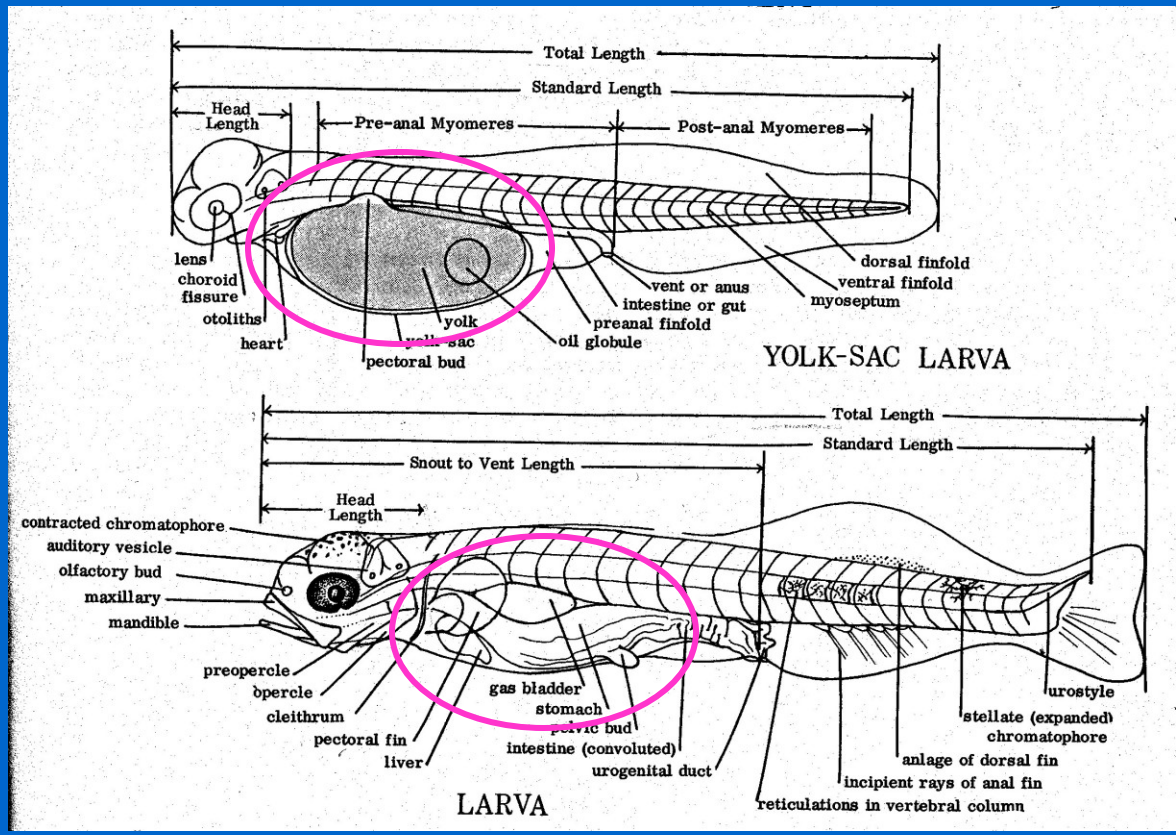
- Tail-bud stage, prominent caudal bulge and cephalic development
- Tail-free stage, separation of the tail from yolk
- Late embryo, embryo has developing characteristics of its hatching stage

Egg Identification

- Translucent or dark
- Buoyant or nonbuoyant
- Adhesive or nonadhesive
- Modifications to aid attachment or flotation
- Spherical or ovoid

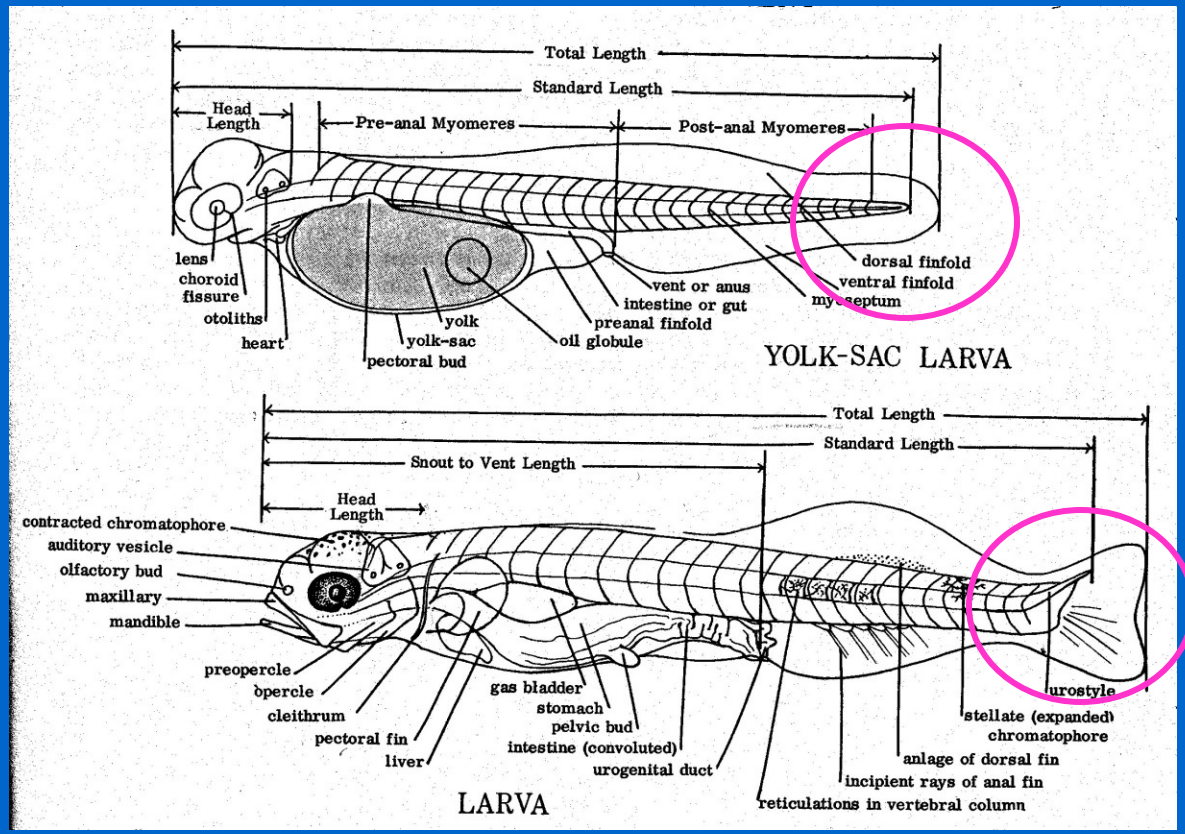
Larval Developmental Stages

- Based on presence or absence of yolk material
  - Yolk-sac larvae
  - Larvae
  - Pre-juvenile or transitional



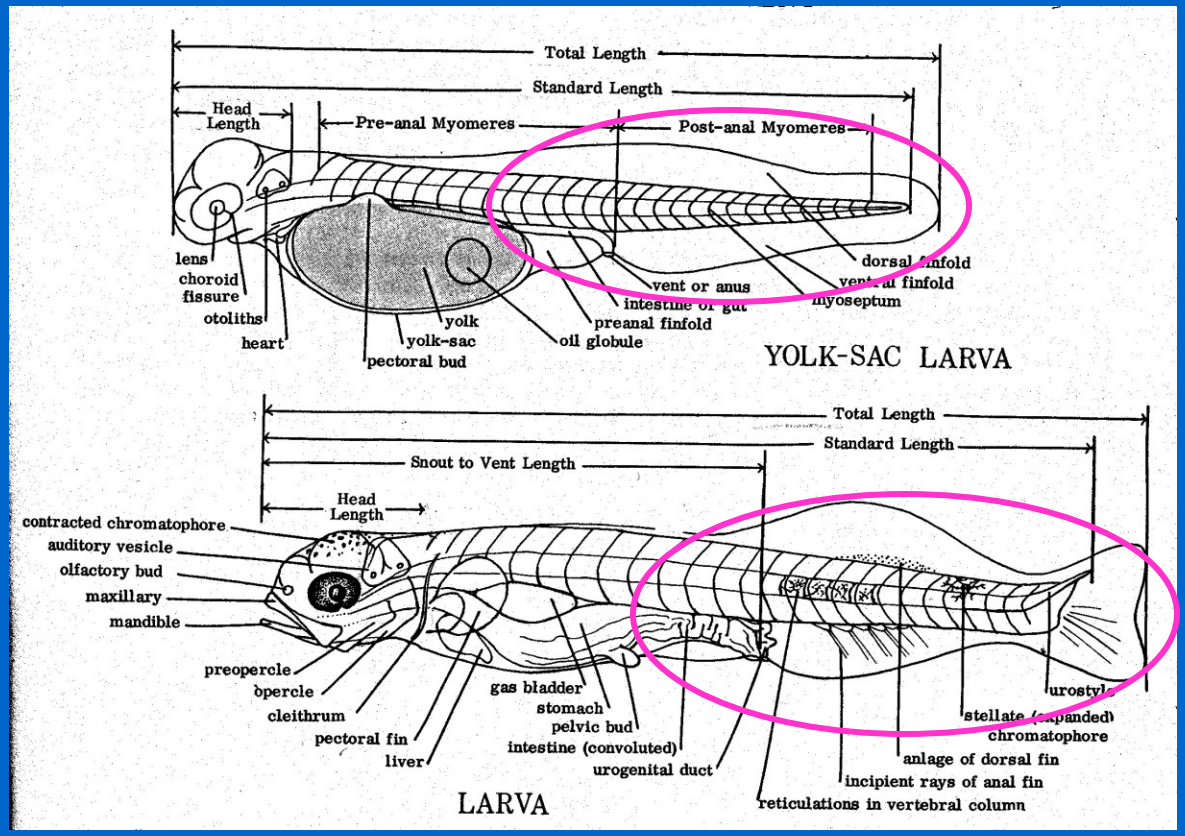
Larval Developmental Stages (cont.)

- Based on changes in the homocercal caudal fin
  - Preflexion larvae
  - Flexion larvae
  - Postflexion larvae

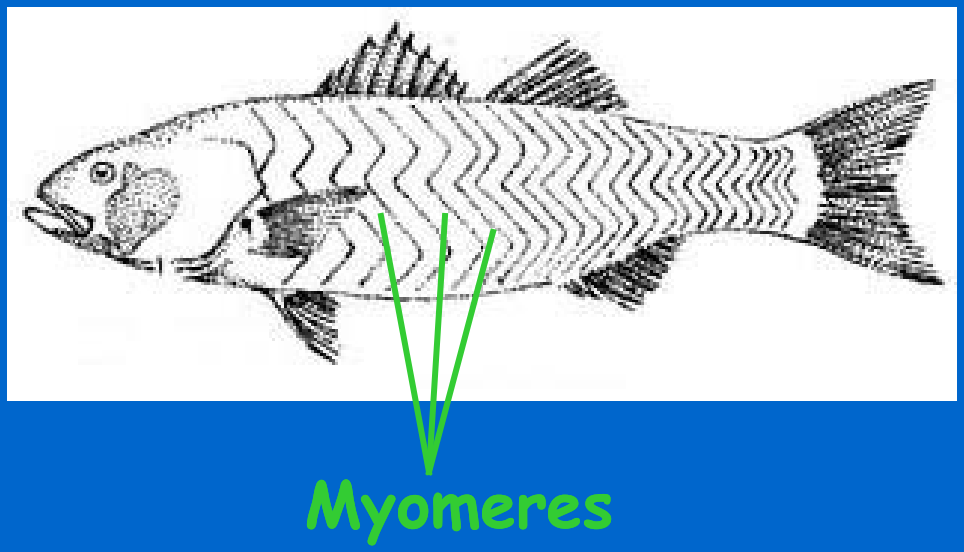


Larval Developmental Stages (cont.)

- Based on morphogenesis of the median finfold and fins
  - Protolarvae
  - Mesolarvae
  - Metalarvae



Larval fish identification



- Several methods of identification
  - Myomere counts
    - Chevron-shaped serial segments of body muscles
  - Morphometric analyses
    - Describe body form

Larval fish identification (cont.)

- Taxonomic guides
- Supplemental identification techniques
  - Osteological features
  - Organism clearing and staining
  - X-ray radiography
  - Histology