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
Marine Systems

- Horizontal and vertical patchiness
- Passive and active aggregation
Vertical patterns of distribution depend on:

- Egg and larval buoyancy
- Larval behavior
- Temperature patterns

[Diagram showing fish distribution in warm and cold regions]
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
• 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 (e.g. 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

- **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
• 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