

**Special Lecture:
How to stain and illustrate
fish larvae**

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Procedure of clearing and staining fish larvae and juveniles

Dingerkus and Uhler (1977)

Needed
chemicals
and goods

Chemicals

- Ethanol
- KOH
- Sodium borate
- Glycerine
- Alcian blue 8GN
- Alizarin red S
- Trypsin
- Thymol

Goods

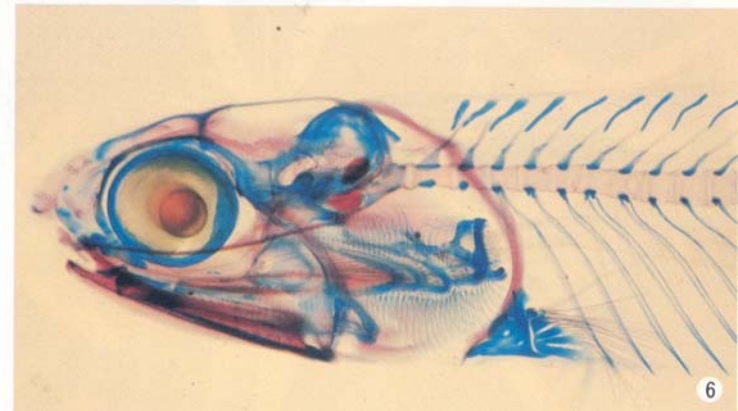
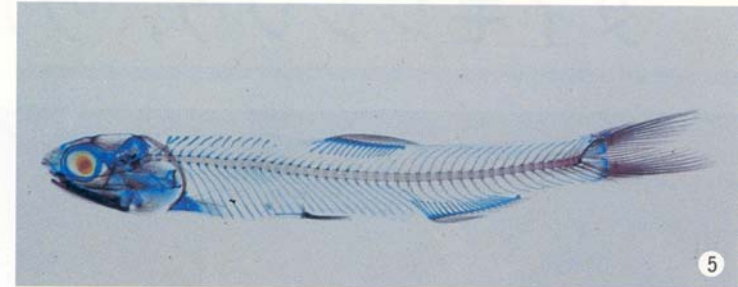
- Laboratory Dishes
- Tweezer

1. Fix fresh material in 10% formalin 2-3 days.
2. Wash in several changes of distilled H₂O, 2-3 days.
3. Place directly into a mixture of 10 mg alcian blue 8GN, 80 ml 95% ethanol, and 20 ml glacial acetic acid, 24-48 hrs.
4. Transfer to 2 changes 95% ethanol, 2-3 hrs in each change.
5. Transfer through 75%, 40%, and 15% ethanol, 2-3 hrs in each, or until specimen sinks.
6. Transfer to distil. H₂O, 2-3 hrs or till specimen sinks.
7. Place in an enzyme solution of 30 ml saturated aqueous sodium borate, 70 distl. H₂O, and 1 g trypsin (4xpancreatin, Nutritional Biochemicals). Change solution every 2-3 days. Continue until bones and cartilage are clearly visible, and flesh retains no blue color.

Dingerkus, G. and Uhler, L. 1977: Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. Stain Technology., 52 (4), 229-232.

Procedure of clearing and staining fish larvae and juveniles (cont'd)

8. Transfer to 0.5% aqueous KOH, to which enough alizarin red S has been added to turn solution deep purple. Leave 24 hrs, or until bones are distinctly red.
9. Transfer through a 0.5% KOH-glycerine series (3:1, 1:1, 1:3) to pure glycerine. To the first two KOH-glycerine solutions, 3 or 4 drops of 3% H₂O₂ may be added per 100 ml solution to bleach pigments of dark specimens. Specimens may be left in the bleaching step for several days or until dark pigment are removed.
10. Store specimens in pure glycerine to which a few crystals of thymol have been added. The thymol inhibits growth of molds and bacteria.



A juvenile of Japanese anchovy (*Engraulis japonica*) cleared and stained. (from Sumikawa and Fujita, 1984)

Needed equipments and goods for illustrating fish larvae and juveniles



Equipments

- Binocular
- Camera lucida
- Measuring apparatus (micrometer)
- Optic and transmitted illuminations for binocular
- Light for drawing paper

Goods

- Pencils (black-2B, red, blue)
- Kent paper
- Tracing paper
- Adhesive tape (or weight)
- Eraser
- *Rotring* pens
- *Maru*-pen
- Cards
- Cut glass in small slip

Procedure of illustrating fish larvae

1. Choosing specimens to be drawn

If possible, 3 specimens of pre-,
flection and post-flection stages

2. Observing, measuring and counting

- TL, FL, SL, HL, BD, ED, SnL, PrAnL, etc
- DF, AF, P1F, P2F, PCR, Br, Myomere
- Pigmentation (by red or blue pencil)
- Spination (location, arrangement)

*should be noted in a card with data of
sampling date (time), position and gear*

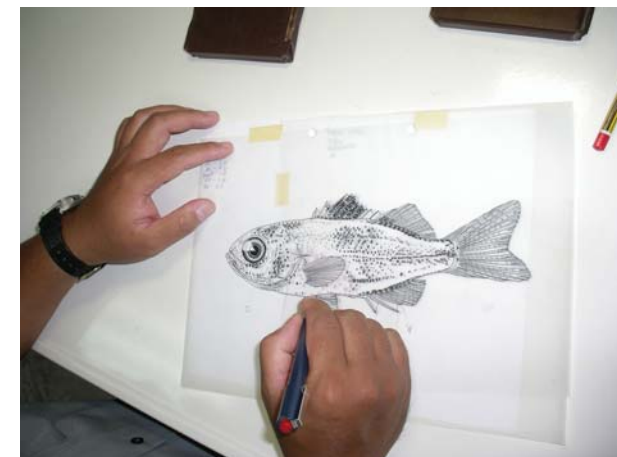
3. Making a first sketch by pencil

on a *kent* paper

- Zoom-up drawing (in full size of A4)
for small-sized larva
- Cut glasses are used to keep the
specimen in a position and to make
twisted body extend.

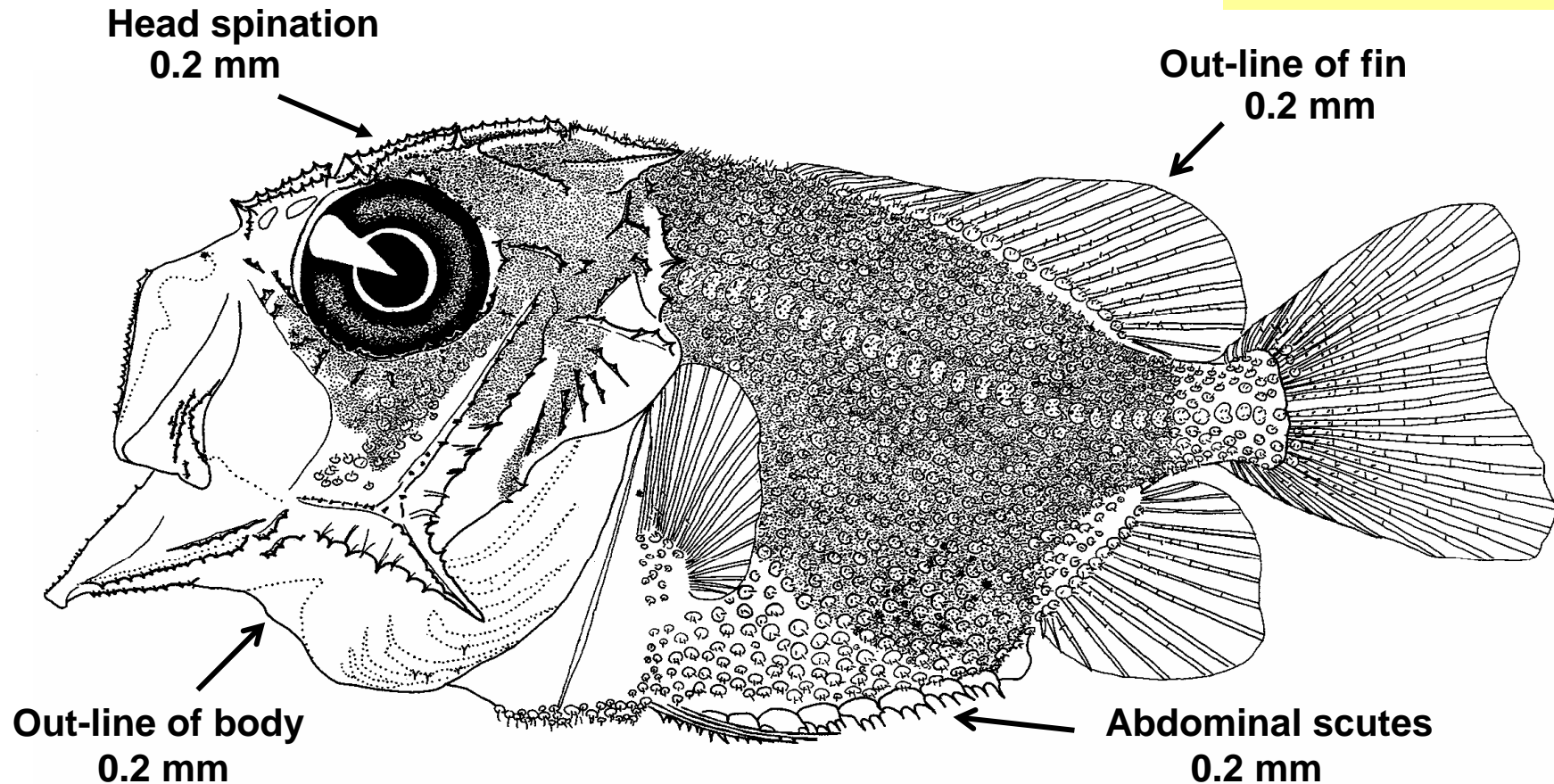
4. Tracing a first sketch covered by a tracing paper with *rotring* pens

If a size of the 1st illustration is larger
than A4, reduce in an A4 size by
a copy machine.



Rotring pens used

Other parts by a
0.1- mm *Rotring*
pen or a *Maru*
pen



***Gephyroberyx japonicus* larva in 11.0-mm SL (Konishi, 1999)**

Konishi, Y. 1999: Developmenta and comparative morphology of Beryciform larvae (Teleostei: Acanthomorpha), with comments on Trachichthyoid relationships. Bull. Seikai Natl. Fish. Res. Inst., 77, 23-92.