

EEB 3898 – Field Methods in Fish Biology – Summer 2014
Protocol for fixation of fish specimens

Materials:

37% Formaldehyde
Sodium Phosphate Monobasic (monohydrate)
Sodium Phosphate Dibasic (anhydrous)
DI Water
70 % ethanol
Glass jars with plastic screw tops

Neutral Buffered Formalin recipe:

1 part formalin
9 parts DI water
Per liter of formalin solution:
4 g Monobasic Sodium Phosphate - Monohydrate
6.5 g Dibasic Sodium Phosphate - Anhydrous

For 20L 10% Neutral Buffered Formalin:

80 g Sodium phosphate monobasic (4g x 20L)
130 g Sodium phosphate dibasic (6.5g x 20L)
2 Liters of 37% Formaldehyde
18 Liters of DI water

Mix solutions well – Good idea to mix the sodium phosphates with a small amount of warm water before adding to the solution.

- 1) Thaw specimen completely on ice or in water
 - a. This takes about 12 hours or more if completely frozen
- 2) Make a small incision in the abdominal cavity to allow formalin solution to bathe the internal organs
- 3) Formalin immersion: Bathe specimen completely in 10% neutral buffered formalin for ~ 2 weeks
- 4) After fixation, rinse the specimen in water for a few minutes under running water
 - a. Do not immerse. This is to remove any excess formalin. You do not want to rid the specimen of all formalin.
- 5) Alcohol step: Place specimen directly in 70% ethanol.