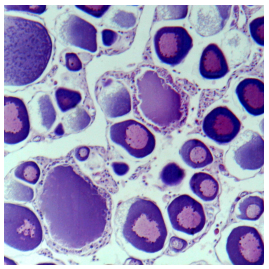
Jun 10, 2019

Histological staining of fish gonadal tissue



DOI
dx.doi.org/10.17504/protocols.io.ygvftw6

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.ygvftw6

Protocol Citation: Jhennifer Gomes Cordeiro, Caio Maximino, Diógenes Henrique de Siqueira-Silva 2019. Histological staining of fish gonadal tissue. **protocols.io https://dx.doi.org/10.17504/protocols.io.ygvftw6**

Protocol status: Working
We use this protocol and it's working

Created: February 23, 2019

Last Modified: June 10, 2019

Protocol Integer ID: 20725

Keywords: Teleost fish, Gonads, Histological staining

Abstract

This protocol describes the dissection, fixation, and histological processing of gonadal tissue of small and medium-sized fish. It has been used in our lab to describe the microanatomy of the gonads of Amazonian Characidae, as well as of ornamental fish such as zebrafish.

Materials

MATERIALS

- Ethanol
- scalpel blades
- 20 mg Eugenol **biorbyt** **Catalog #**orb104769
- Tweezers
- Sterile glass slides
- Toluidine blue O **Bio Basic Inc.** **Catalog #**TB0962.SIZE.5g
- Xylene **Bio Basic Inc.** **Catalog #**XC9800.SIZE.1L
- Embedding base molds **Fisher Scientific** **Catalog #**22-363-553
- Standard square cover slips 18×18 mm
- Surgical scissors (Iris - straight) **Braintree Scientific** **Catalog #**SCT-I 528
- Hematoxylin **Sakura Finetek**
- Eosin **Sakura Finetek**
- Bouins Fixative **Polysciences, Inc.**
- 25 ml glass bottles
- Glycol methacrylato resin
- Resin hardener powder
- Epoxy glue **Tekbond**
- Tabletop drying oven **Thermo Fisher Scientific**
- Rotary microtome **Leica Biosystems**
- Permount mounting medium **Fisher Scientific**

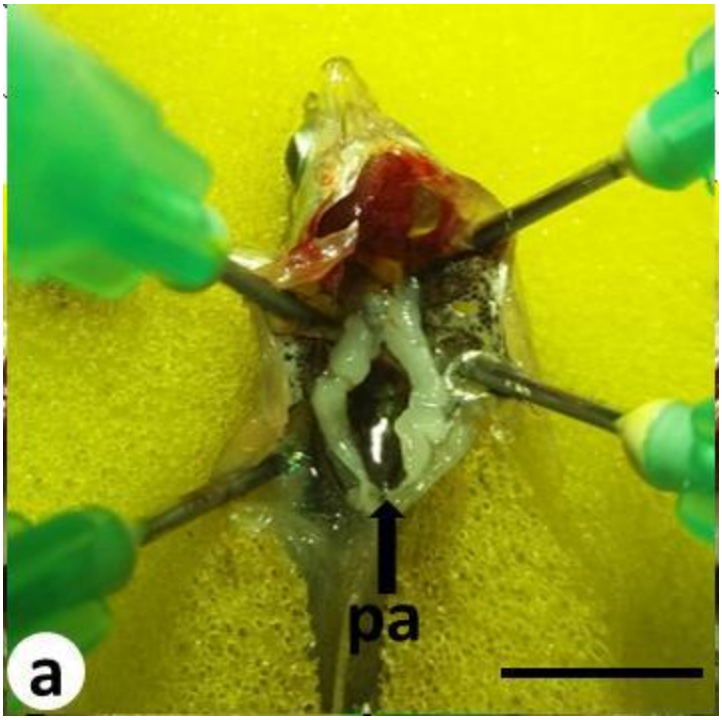
Safety warnings

Some of the reagents used, including Bouin's Fixative, may cause serious skin and eye damage, and may cause cancer if inhaled. Wear eye protection, face protection, protective clothing, and protective gloves when manipulating these reagents.

Collection and fixation of gonads

1 Anesthetize and euthanize the animals

2 Perform a ventro-longitudinal incision to expose the gonads, Bouin's fixative must be immediately applied on the gonads aiming the prefixation. Then, gonads are removed and immediately placed in the fixative



3 2Leave gonads overnight in Bouin's fixative for 24h 24:00:00

4 After 24h, the fixative solution must be replaced with 70% ethanol, that should be renewed every day until there is no trace of fixative

Dehydration

5 Before dehydration the tissue must be sectioned in the desired shape and fit into the molds

6 Samples need to be placed in glass vials, to which the solutions will be added

7 Transfer the samples to three fast changes of 70%, 80%, and 90% ethanol
00:03:00 for each change

8 Leave the samples at 95% ethanol for 40 min. 00:40:00

Inclusion

9 Include samples in 1:1 solution of 95% alcohol and infiltration resin (Methacrylate glycol) for 40 minutes 00:40:00

10 Leave samples in infiltration resin (methacrylate glycol) "overnight" (or at least 12 hours)
12:00:00

11 Prepare the inclusion resin solution (Methacrylate glycol) with the hardener (for each 1.0 mL of resin use 0.07 mL of hardener)

12 Embedding the material: place the material on the mold and place the resin + hardener solution on it by completing the mold

13 After the resin polymerizes, leave embedded samples in a drying stove for 24 h
24:00:00

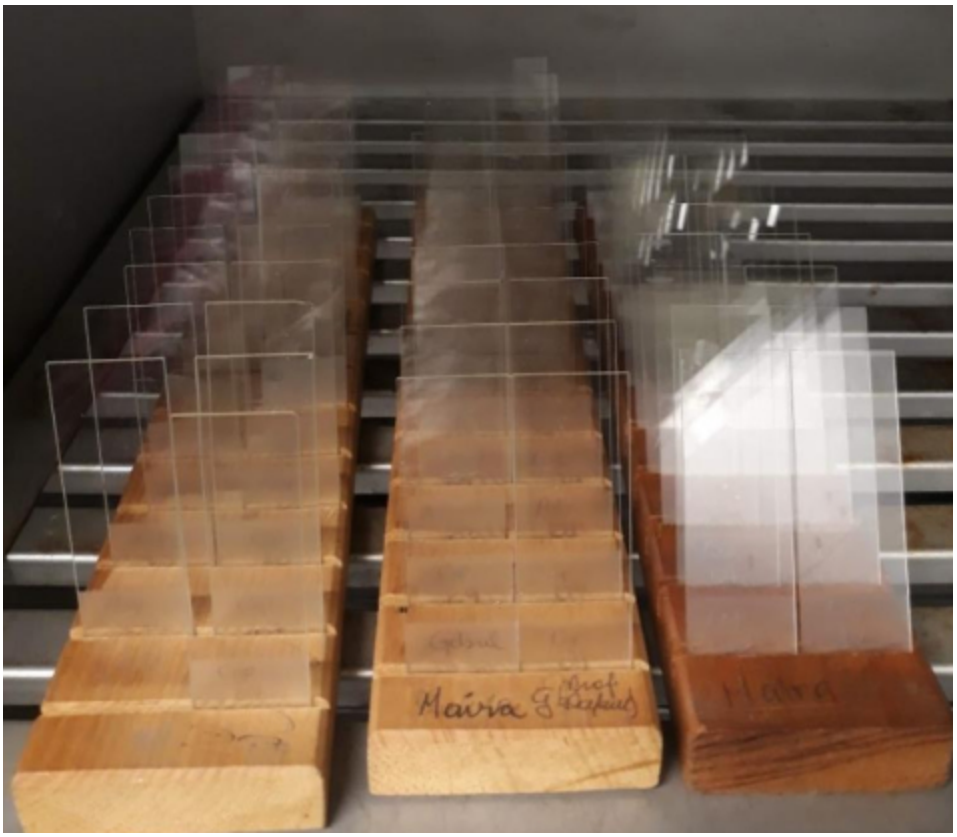
14 Unblock and glue on microtome support stick with epoxy resin glue

15 After the glue has completely dried, the sample should be taken to the microtome

16 The sample should be sectioned to the thickness of 3 to 5 µm

17 The cuts should be deposited on properly identified slides

18 The slides should be kept in the drying stove for 1 day and then followed for staining procedures. If staining with H&E, follow steps 19-27. If staining with toluidine blue, follow steps 28-33 24:00:00



Staining: Hematoxylin and eosin

19 Moisturize cuts in running tap water for 10 minutes 00:10:00

20 Leave in Harri's hematoxylin solution for 15 minutes 00:15:00

21 1Wash in running tap water for 10 minutes 00:10:00

22 Dehydrate in 90% alcohol with fast change (if eosin is alcoholic) 00:03:00

23 Leave in eosin solution for 15 minutes 00:15:00

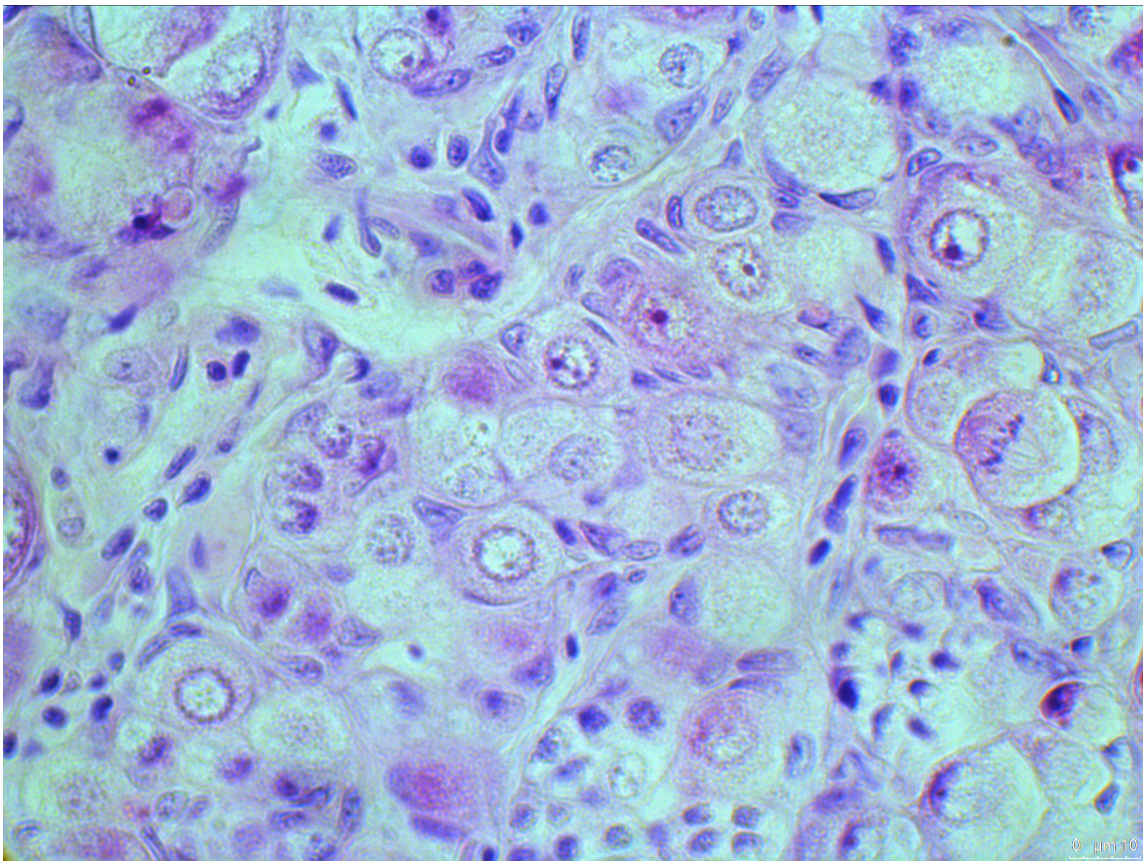
24 Rinse well under running water

25 Transfer the samples to three fast changes of 70%, 80%, and 95% ethanol, followed by fast passes in absolute ethanol, and two changes of xylene (3 min for each station) for clearing before coverslipping 00:03:00 for each change

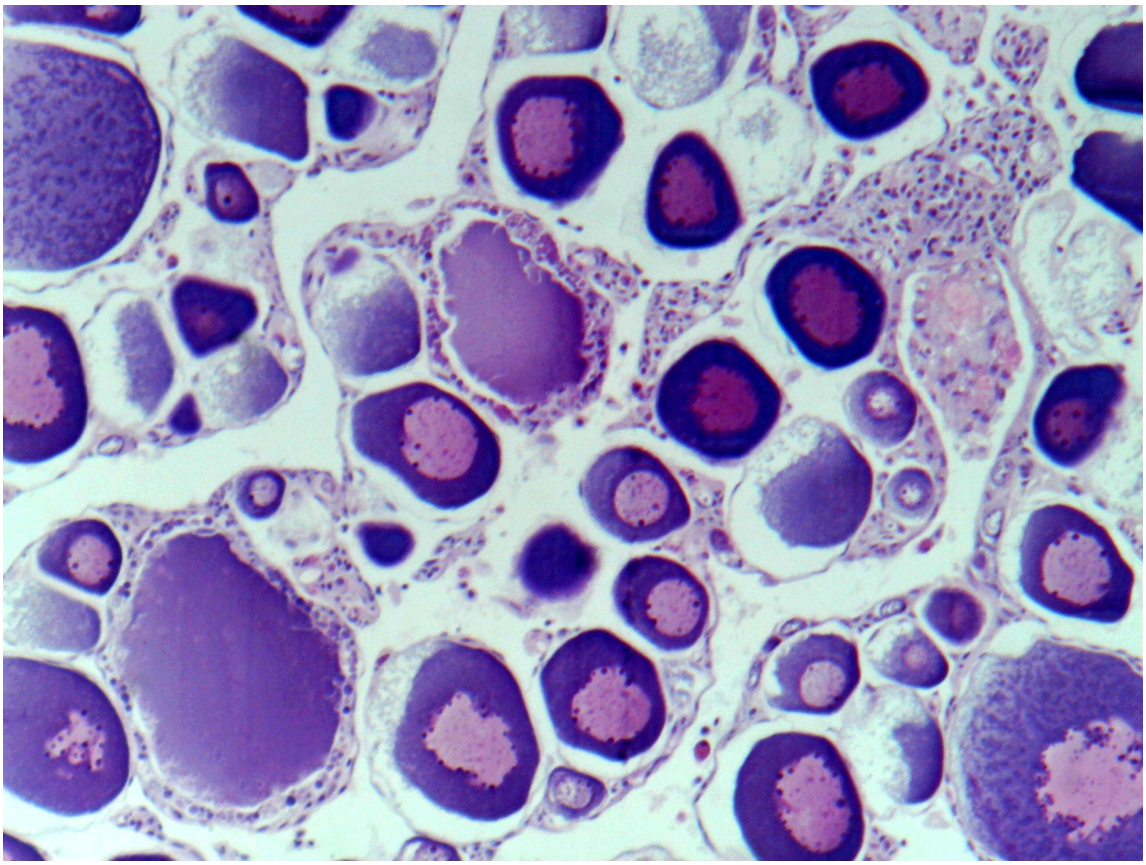
26 Transfer the sample to a third change of xylene 00:03:00

27 Mount and coverslip in Permount (xylene-based mounting medium)

28 Check slides on the microscope and take photographs as needed.
Example slides:



Immature female *Astyanax bimaculatus* gonads



Initial gonadal development in male *Astyanax bimaculatus*

Staining: Toluidine blue

29 Moisturize cuts in running water for 10 minutes 00:10:00

30 Leave in toluidine blue solution for 15 minutes 00:15:00

31 Wash in running water for 10 minutes 00:10:00

32 Transfer the samples to three fast passes of 70%, 80%, and 95% ethanol, followed by fast passes in absolute ethanol, and two changes of xylene (3 min for each station) for clearing before coverslipping 00:03:00 for each change

33 Transfer the sample to a third change of xylene 00:03:00

34 Mount and coverslip in Permount (xylene-based mounting medium)