JUN 10, 2019

Histological staining of fish gonadal tissue

Jhennifer Gomes Cordeiro¹, Caio Maximino¹,
Diógenes Henrique de Siqueira-Silva¹

¹Universidade Federal do Sul e Sudeste do Pará

Fish behavior and physiology

Medicinal Plants Southeastern Pará Research Group

Caio Maximino

Universidade Federal do Sul e Sudeste do Pará

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocol.s.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/protocol.s.io.ygvftw6>

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

Created: Feb 23, 2019

Last Modified: Jun 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

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- Ethanol **Contributed by users**
- scalpel blades **Contributed by users**
- 20 mg Eugenol **biorbyt Catalog #orb104769**
- Tweezers **Contributed by users**
- Sterile glass slides **Contributed by users**
- 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 **Contributed by users**
- Surgical scissors (Iris - straight) **Braintree Scientific Catalog #SCT-I 528**
- Hematoxylin **Sakura Finetek**
- Eosin **Sakura Finetek**
- Bouins Fixative **Polysciences**
- 25 ml glass bottles **Contributed by users**
- Glycol methacrylate resin **Contributed by users**
- Resin hardener powder **Contributed by users**
- 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 Leave 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)