

Supplementary materials

Herein, we provide a detailed description of the solutions and protocol for the staining method that includes a combination of periodic acid Schiff's and Mallory's trichrome. The description was provided by P. Witthames, and the method was applied in Witthames and Greer Walker (1995) and in Witthames et al. (2010).

Preparation of special solutions

Periodic acid Schiff's

Quantity	Item and comment
200 mL	Reverse osmosis water—heat to just off boiling, stir, and add
1 g	Pararosaniline
2 g	Potassium metabisulphite—after cooling to 50°C, stir
2 mL	Concentrate 35% HCl—add after cooling to room temperature
2 g	Activated charcoal overnight in a stoppered flask Filter through a Whatman no. 1 filter. Stable when refrigerated for several weeks. Discard when pink. May be used several times.

Mallory's trichrome

Quantity	Item and comment
200 mL	Reverse osmosis water
1.0 g	Aniline blue
4.0 g	Orange <i>G</i>
4.0 g	Ocalic acid

Protocol

Step	Solution or action	Time
1	5% periodic acid	4.5 min
2	Wash 5 times thoroughly with distilled water	–
3	Schiffs reagent	60 min
4	Wash running tap water	10 min
5	1% acid fuchsin	1 min
6	Wash distilled water	30 s
7	Wash distilled water	30 s
8	1% phosphomolybdic acid	1 min
9	Wash distilled water	10 s
10	Mallory's trichrome	15 s
11	Distilled water	10 s
12	90% industrial methylated spirits (IMS)	5 s
13	100% IMS	5 s
14	100% IMS	5 s
15	1:1 ratio of IMS to CitrocLEAR	5 s
16	CitrocLEAR	5 s
17	CitrocLEAR	5 s
18	Mount with DPX, dry at 40°C overnight	–

References

Witthames, P. R., and M. G. Walker

1995. Determinacy of fecundity and oocyte atresia in sole (*Solea solea*) from the Channel, the North Sea and the Irish Sea. *Aquat. Living Resour.* 8:91–109.

<https://doi.org/10.1051/alr:1995007>

Witthames, P. R., A. Thorsen, and O. S. Kjesbu.

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