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Information on microscopical methods

A method for fixing and staining peritrich ciliates

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Summary

A simple technique for fixing and staining peritrich ciliates involving fixation in phosphate buffered formalin and staining with Sémichon's acetic-carmine is presented. This procedure appears to be superior to conventional methods for achieving relaxation of zooids and for differentiation of the macronucleus.

Eine Methode zum Fixieren und Färben von rundum begeißelten Ciliaten

Diese einfache Technik des Fixierens und Färbens von allseitig begeißelten Ciliaten umfaßt die Fixation in mit Phosphat gepuffertem Formalin und die Färbung in Essigsäure-Karmin nach Sémichon. Im Vergleich mit üblichen Verfahren scheint diese Methode vorteilhafter zu sein zur Entspannung der Zooide und zur Differenzierung des Makronukleus.

Our ongoing investigation of the epizootiology of a disease involving the bacterium *Aeromonas hydrophila* and the peritrich ciliate *Epistylis* sp. among centrarchid fishes in a South Carolina cooling reservoir (Esch et al. [1]) necessitated fixing in the field, the characteristic lesions produced by this disease. Several attempts with well established fixation methods failed to produce sufficiently well relaxed and expanded *Epistylis* colonies to allow subsequent examination of the preserved specimens. A suitable method to achieve the desired relaxation and fixation is herein described.

Pennak [5], Kudo [3] and Humason [2] suggest that either Bouins or FAA (Formalin-Acetic Acid-Alcohol) can be used as a fixative for ciliated protozoa. However, we have found that the use of either of these fixatives resulted in such contraction of the zooids that poor stain penetration was obtained and it was impossible to observe the oral ciliature (Fig. 1). Neither heating nor cooling these fixatives prior to use significantly alleviated the contraction problem.

In contrast to the results obtained with either Bouins or FAA, we have had considerable success in fixing several peritrichs with phosphate buffered formalin

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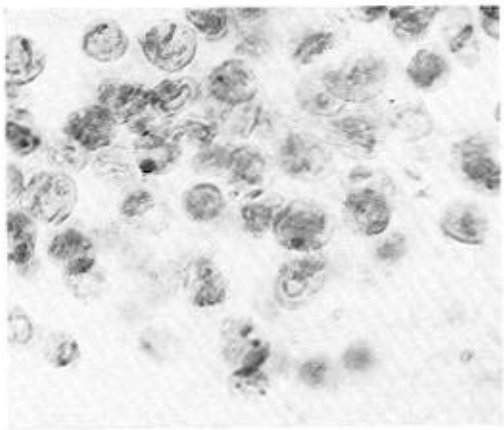

Fig. 1: Scrapings of *Micropterus salmoides* lesion. Fixed with Bouins and stained with Sémichon's acetic-carmine stain (Scale 250:1).

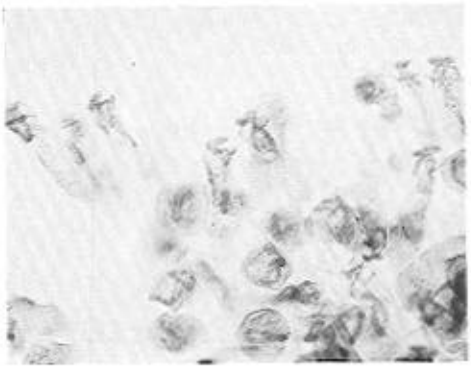

Fig. 2: Scrapings of *Micropterus salmoides* lesion. Fixed with phosphate buffered formalin and stained with Sémichon's acetic-carmine stain (Scale 250:1).

(pH 7.0), (Humason [2]). This fixative resulted in good relaxation of the zooids which permits observation of the oral ciliature and effects better stain penetration (Fig. 2).

Delafield's hematoxylin and several vital stains (e. g. neutral red, methylene blue, toluidine blue) have been recommended for staining various protozoa (Pennak [5]; Kudo [3]; Humason [2]). However, we have found that these stains resulted in a limited ability to differentiate the macronucleus from the cytoplasm of the peritrichs we have examined. We have achieved much better resolution of the macronucleus of *Epistylis* by staining with Sémichon's acetic-carmine (Meyers and Olsen [4]) followed by destaining in acid alcohol, dehydration and clearing in xylene (Fig. 2).

This combination of techniques involving fixing and relaxation with phosphate buffered formalin and subsequent staining with Sémichon's acetic-carmine has proven to be effective in our investigation of the lesions typical of the disease involving the *Aeromonas-Epistylis* complex. The techniques should have broader application in the study of other ciliates, since we have achieved similar results with *Vorticella* spp. and *Zoothamnium* sp.

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