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Use of fluorescence to find parasitic nematodes

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Introduction

Many living and dead animal tissues show visible fluorescence when irradiated with ultraviolet light (Encyclopedia Britannica). Ortolani and Campanile (1966) used this characteristic in general epidemiological surveys for vaginal trichomoniasis and found that examination of fresh material with a fluorescence microscope was superior to standard microscope inspection after staining. Other workers, studying bacteria (Pulvertaft, 1934; Slavnina, 1948; Wasserman, 1965) and lichens (Cernohorsky, 1950 and 1959), have demonstrated that the colour of fluorescence may be dependent on the species or strain of organism involved. In this paper the use of autofluorescence to help find and identify parasitic nematodes *in situ* is discussed.

Methods and materials

Fluorescence characteristics of the following parasitic nematodes were determined: larval and adult *Contracaecum aduncum* and larval *Anisakis* sp. from Atlantic salmon; adult *Anisakis* sp. and *Porrocaecum decipiens* from grey seals; larval *P. decipiens* from Atlantic codfish; and adult *Philonema agubernaculum* from Arctic char. Except for larval *Anisakis*, which were examined before and after freezing, all examinations were made with previously frozen material. Host tissues and organs harbouring parasites were irradiated with ultraviolet light of 360 mμ (Fisher Multi-Ray Lamp No. 11-988V1 with No. 11-988-4 long wave light). Specimens were viewed through UV protective goggles (Fisher No. 11-403) to eliminate reflected UV and near UV blue light.

Results and discussion

All species tested, except *P. agubernaculum* fluoresced. Live *Anisakis* had a very pale fluorescence but specimens frozen before examination fluoresced brilliantly. This change in fluorescence is similar to Manohar's (1969) finding that the intensity of fluorescence of fish muscle after freezing is always greater than that before freezing. The following table illustrates the fluorescence characteristics of the nematodes

examined:

Larval and adult *Anisakis* - brilliant bluish-white

Larval and adult *C. aduncum* - pale to bright yellow

Larval *P. decipiens* - brilliant bluish-white

Adult *P. decipiens* - variable: pale to bright yellow to pale to bright bluish-white possible in different areas of the same specimen.

When two species with different fluorescence characteristics were found in a single sample of hosts (*Anisakis* sp. and *C. aduncum* only were present in the body cavity of the salmon examined) immediate preliminary identification and sorting of the specimens was possible. All the nematodes fluoresced much brighter and with a different colour than the surrounding tissues (seal stomachs, orange; salmon viscera, red to pale yellow). Thus, the nematodes were readily visible under UV light even when only a fraction of a millimetre of their body length was in view. Because UV light does not penetrate deeply in animal tissues and the intensity of light emitted by the worms is low, nematodes embedded more than about 0.5 mm below the tissue surface are not visible. Consequently, this method cannot be used to find nematodes in fish muscles (in this respect, incident white light (Power, 1958) is superior).

Since some parasitic nematodes, such as larval *Anisakis* and *Contracaecum*, are small and almost invisible against the background of hosts' tissues accurate counts are difficult and time consuming. However, when irradiated with UV light all the parasites were quickly located. In an experiment involving examination of viscera from two samples of 11 salmon each, the same examiner found less than 26% of the worms in the first six minutes of searching with visible light but all the worms in the same time period using UV light (Fig. 1). When working with UV light examiners were confident that they had found all the worms present. However they were never confident when using visible light. The use of fluorescence to find parasitic nematodes seems particularly well suited for long-term investigations or where accurate counts are necessary.

Examinations were carried out in the lighted laboratory or in the field by the use of a reasonably light-proof chamber with the UV light source mounted inside.

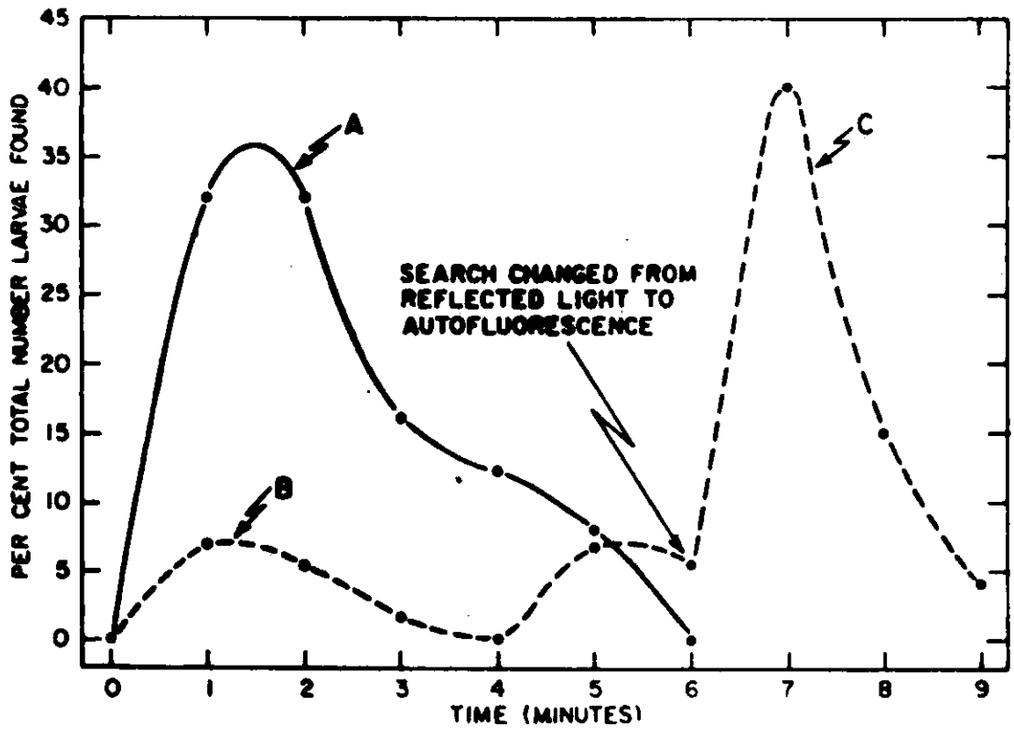

Fig. 1. Relative efficiency of finding *Anisakis* larvae on the viscera of Atlantic salmon by searching with: A. autofluorescence caused by ultraviolet light (total 25 larvae found), and B. reflected visible light. Curve C shows additional larvae found when viscera used in B were examined under ultraviolet light for an additional three minutes (total 57 worms for curve B-C).

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