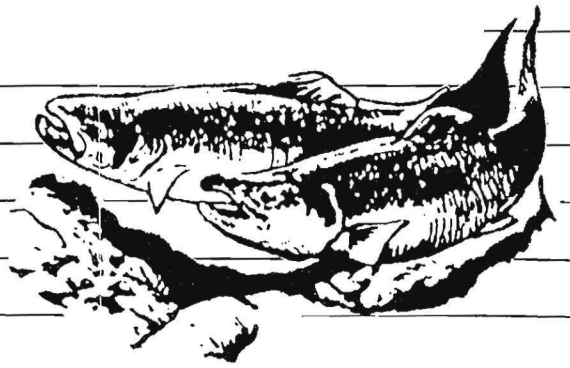
Research



Information bulletin

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Water Sampling Technique Detects Bacterium That Causes Columnaris Disease in Fish

Columnaris disease, caused by *Cytophaga (Flexibacter) columnaris*, affects salmonids and most warmwater fishes throughout the world. Infected fish often have extensive gill necrosis, and columnar aggregates ("haystacks") of bacteria are revealed by microscopic examination of the gills. Necrotic lesions are common along outer margins of fins and over the dermis. This bacterium is isolated on specialized media, and other microbes can overgrow the pathogen. The disease is controlled by administration of oral antibiotics or bath treatments of potassium permangenate. Both treatments have several disadvantages: (1) sensitivity of this bacterium to antibiotics is difficult to assess, (2) potassium permangenate can exacerbate existing lesions, and (3) to date, neither treatment is legal. In fact, no drugs or chemicals are legally available to treat food fish for columnaris disease.

At present, the best method of controlling columnaris disease in fish culture facilities is prevention. Although data concerning transmission and epidemiology of the disease are limited, *C. columnaris* can exist in water for as long as 32 days. Detection of the organism in water samples would alert hatchery personnel to make informed management decisions.

Water Filtration Method

The water filtration method is similar to the procedure used to detect *Aeromonas salmonicida*. Briefly, the assay is conducted as follows:

1. Prepare Tryptone-Yeast-Gelatin (TYG) agar plates by adding 2 g tryptone, 0.5 g yeast extract, 2 g gelatin and 15 g agar to 1 L of distilled water. Autoclave and dispense into sterile petri dishes.

2. Collect and pass water through a Millipore filter apparatus using a presterilized 0.45 µm filter. The water sample may be diluted before filtering if necessary for accurate bacterial counts.

3. Blot the filter (grid-side down) onto TYG agar for approximately 5 min. Remove and discard the filter.

4. Incubate TYG plates for at least 96 h at room temperature. Calculate the number of colony-forming units (cfu)/mL of water for each sample. Transfer yellow-rhizoid colonies to Tryptone-Yeast Extract-Salts (TYES) broth for further identification. TYES broth is 4 g tryptone, 0.4 g yeast extract, 0.5 g $MgSO_4*7H_2O$ and 0.5 g $CaCl_2*2H_2O$ in 1 L distilled water.

Detection of *Cytophaga columnaris* at a Broodstock Facility

In June and July 1993, water samples plated on TYG by trained personnel at the Richard Cronin National Salmon Station, Sunderland, Massachusetts, were shipped to the National Fish Health Laboratory, Kearneysville, West Virginia, and further processed. After 96 h incubation, yellow rhizoid colonies typical of *C. columnaris* were transferred to TYES broth. Each isolate was screened for characteristics including growth on medium containing neomycin sulfate and polymyxin B, production of gelantinase, binding of Congo red dye and production of chondroitinase. Isolates positive for these characteristics were identified as *C. columnaris.*

Cytophaga columnaris was found during the 1993 spawning season in effluent from all tanks containing adult, sea-run Atlantic salmon. Bacterial concentrations of *C. columnaris* in these tanks were between 0.01 cfu/mL and 1.0 cfu/mL. Because salmon in these tanks did not show any signs of clinical disease, concentrations of *C. columnaris* were not indicative of bacterial densities expected during epizootics.

Advantages

Monitoring concentration of *C. columnaris* in effluent water from fish culture facilities provides valuable data concerning transmission and epidemiology of columnaris disease. The method described is simple, inexpensive, and easily performed by hatchery personnel. After further testing, this method may provide a reliable means of detecting *C. columnaris* before the onset of disease. The assay may also be useful in monitoring efficacy of treatments.

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