

Sterility of Salmonid Roe and Practicality of Hatching Gnotobiotic Salmonid Fish

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The ventral surface of spawning salmonid fish was opened aseptically and the roe were removed aseptically. Roe obtained by using this technique were demonstrated to be sterile. Aseptic fertilization and incubation of eggs obtained in this manner resulted in the hatching of gnotobiotic salmonid fish.

Gnotobiotic animals have provided useful models in studies of host-parasite relationships in a number of homeothermic species (2, 4). Fish have attracted little attention aside from the work of Baker and Ferguson (1) on the platyfish (*Platypecilus maculatus*). It is particularly unfortunate that salmonid fish have not received consideration as a potentially useful model for germfree and defined flora studies in poikilothermic species, especially since a spawning female can produce as many as 4,000 ova (3). This paper is intended to demonstrate the ease with which these ova can successfully be collected, fertilized, incubated, and hatched aseptically.

MATERIALS AND METHODS

Sources of roe. Sexually mature fish were caught by net and transported to the laboratory in ice-packed polystyrene containers. Coho salmon (*Oncorhynchus kisutch*) were obtained from the Chemainus River, chum salmon (*Oncorhynchus keta*) were from the Goldstream River, and the kokanee salmon (*Oncorhynchus nerka*) were from the Okanagan River. The golden trout (*Salmo aquabonito*) were obtained from Beatty Lake, the rainbow trout (*Salmo gairdneri*) were from the British Columbia Department of Recreation and Conservation Hatchery at Richmond, and the brook trout (*Salvelinus fontinalis*) were from Aylmer Lake, British Columbia.

Aseptic collection of roe. The ventral surface of the fish was thoroughly scrubbed with povidone-iodine disinfectant (Bridine; British Drug Houses) containing 1% available iodine. Standard aseptic procedures were used to open the ventral wall of the fish body and expose the peritoneal cavity. Milt was removed with a sterile Pasteur pipette, and sections of testes were cut and transferred to sterile containers. Ova were removed with a sterile spoon-shaped spatula.

Aseptic incubation of fertilized eggs. Eggs of three fish species were fertilized aseptically, washed free of milt, and incubated at 13 C in anaerobic bacterial culture dishes. Each dish contained 25 eggs

in 100 ml of sterile well water (5). Incubation water was changed aseptically at 2-day intervals, and 1-ml samples were removed to detect bacterial contamination.

Sterility testing. To test for sterility, samples of ova, milt, and incubation water were transferred to 10 ml of sterility test broth, brain heart infusion broth, Trypticase soy broth, or glucose fermentation broth (Baltimore Biological Laboratories) and were incubated aerobically and anaerobically at 13 and 30 C. After 21 days, tubes were examined for turbidity and plated on sterility test agar, and the plates were incubated under the appropriate conditions for a further 10 days.

RESULTS AND DISCUSSION

A total of 200 ova from two coho salmon, 300 ova from two golden trout, 200 ova from two kokanee salmon, and 1,000 ova from four rainbow trout failed to yield bacterial growth. Similarly, 100 samples containing 0.25 to 1 ml of milt from two chum salmon, 150 milt samples from three coho salmon, and 100 milt samples from two brook trout failed to yield bacterial growth. In addition, five sections of golden trout testes, 15 samples of testes from four brook trout, and whole testes from ten rainbow trout were demonstrated to be free from bacterial contamination. To demonstrate that media which contained roe were capable of supporting bacterial growth, a control set of media containing eggs or milt was inoculated with 10^8 viable cells of *Aeromonas liquefaciens* ATCC 14715, *Aeromonas salmonicida* ATCC 14174, *Chondrococcus columnaris* ATCC 23463, *Escherichia coli* ATCC 11775, *Micrococcus lysodeikticus* ATCC 4698, *Pseudomonas aeruginosa* ATCC 15442, *Pseudomonas fluorescens* ATCC 13525, *Pseudomonas putida* ATCC 12633, or *Staphylococcus aureus* ATCC 6538. All organisms grew in the presence of roe.

An additional 100 eggs of three fish species

were successively fertilized and incubated aseptically for 40 days, by which time the embryo was well developed. Control batches of eggs maintained under normal hatchery conditions contained as many as 10^7 viable bacteria per cm^2 of surface (5). All eggs save five coho eggs, five chum eggs, and 25 kokanee eggs were tested for bacterial sterility and confirmed by incubation of the eggs in sterility test broth. The remaining 35 embryonated eggs were allowed to develop, and all eggs had hatched after 60 days. These fish were successfully maintained for an additional 61 days, by which time the yolk sac had been consumed. The bacterial sterility of these alevins was confirmed after incubation in sterility test broth failed to yield bacterial growth. A control population of alevins at the yolk sac stage maintained under normal hatchery conditions contained from 10^4 to 10^8 viable aerobic bacteria per g (wet weight) of fish when assayed by the procedures of Trust (5).

The success obtained by using these simple techniques suggests that with little additional equipment it should be possible to raise large numbers of germfree salmonids to physical maturity. A satisfactory method for the sterilization of fish diets has already been reported (6). The use of these simple procedures has other applications. The techniques are completely feasible for the establishment of specific

pathogen-free egg and fish populations, as well as known flora egg and fish populations. In addition, since the technique can be extended to handle large numbers of spawning males and females and hence yield large numbers of fertilized eggs, it eliminated or reduces the need to surface-disinfect eggs before intra- or international shipment. The technique also reduces the chances of the transfer of infectious agents which may not be destroyed by surface disinfection.

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