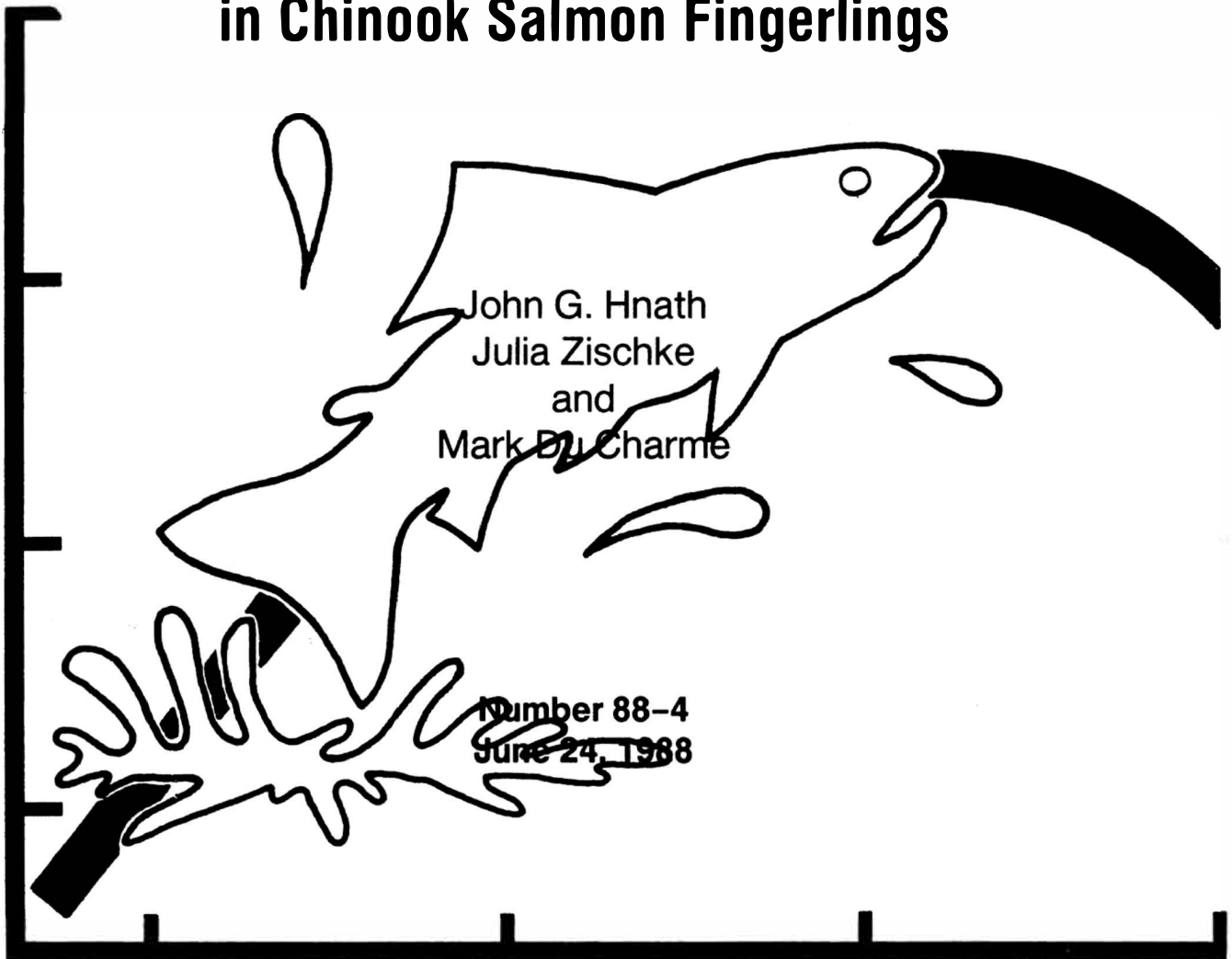


FISHERIES DIVISION

TECHNICAL REPORT

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Michigan Department of
Natural Resources

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**ATTEMPTS TO DETECT ENTERIC REDMOUTH (ERM)
IN CHINOOK SALMON FINGERLINGS¹**

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¹A contribution from Dingell-Johnson Project F-35-R, Michigan

INTRODUCTION

Enteric redmouth (ERM) caused by *Yersinia ruckeri* is a serious bacterial infection of trout, particularly severe in rainbow trout. Historically, this pathogen has not been found in Great Lakes waters although it has been isolated sporadically from areas within the basin. For the past 2 years the pathogen has been isolated from chinook salmon fingerlings being reared at State of Illinois hatcheries. The fish originated both years as eggs from Lake Michigan spawners, the same stock from which Michigan takes its eggs. However, to date there has been no isolation of this pathogen from either adult chinook or chinook fingerlings in Michigan. The adult chinook, returning from Lake Michigan to the Little Manistee spawn-taking weir, have been inspected for disease since 1971 without a single isolation of ERM. For many of these years the chinook were additionally sampled and inspected by the State of Illinois, and no ERM was ever isolated. Since the disease was detected in Illinois chinook of Lake Michigan origin, production fish in Michigan have been intensively inspected for the pathogen using brain heart infusion (BHI) agar streaks from kidney, liver, and gut from large numbers of samples. Sample sizes were as follows:

Location	Year	
	1986	1987
Wolf Lake Hatchery	117	48
Platte River Hatchery	20	60
Thompson Hatchery	20	114
Little Manistee Weir	32	—
Platte River Weir	13	—
Total	202	222

In addition to the above samples from chinook salmon, all salmonid species in State of Michigan hatcheries have been inspected each year for diseases (as has been the practice since 1971) and no isolate of ERM or ERM-like organisms has been found.

Since it is important to know the source of ERM in Illinois chinook, the State of Illinois did in-depth testing of all possible sources of infection, with no conclusive results. Thus, we felt it important to see if our production chinook might be asymptomatic carriers of infection. Since ovarian fluids of spawning chinook were 100% positive for bacterial kidney disease (BKD), although no overt clinical signs of the disease were observed, we were also concerned that the progeny might serve as carriers of this pathogen. Our ultimate concern was that if the chinook fingerlings were carriers of either or both of the pathogens, could they then serve to

transmit the infection(s) to other fish at hatcheries where they are reared? This study was initiated to try to answer these questions.

METHODS

One thousand chinook fingerlings from the Wolf Lake Hatchery production lot were placed into each of six tanks at the fish health laboratory. Into the foot of each tank were also placed 200 brook trout fingerlings from the indoor rearing tanks at Oden Hatchery. Two tanks were set up as controls with >8 ppm dissolved oxygen (DO) in effluent, two with <5 ppm DO, and two with <5 ppm DO and nitrogen supersaturation. All mortalities were monitored for BKD using the fluorescent antibody technique (FAT) on kidney, spleen, and liver homogenates, and for ERM using BHI agar streaked from the same homogenates and standard biochemical methods. It was recommended by Rod Horner (State of Illinois Fish Pathologist) that we provide a serious DO stress initially and again a week later by turning the water off until the DO dropped to 2.0 ppm and the fish showed severe stress. This he assured us would cause a disease response within 2 weeks.

Fish were reared for 54 days, although 30 days were considered adequate to see the effects of stress-induced disease.

Dissolved oxygen stress was applied to two tanks weekly by turning the water flow off until the fish showed severe stress and started turning "belly up". This took from 50 to 110 minutes, during which time DO levels dropped from 9.5 to 2.6 ppm.

Nitrogen gas supersaturation stress was obtained by pressurizing incoming water with an air leak on the pump intake line. Gas levels had diurnal fluctuations. From 8:00 A.M. to 4:30 P.M. gas levels were 107–112% total dissolved gas (TDG), 8.4–8.8 ppm DO, and 116–121% nitrogen saturation. From 4:30 P.M. to 8:00 A.M. and all day Saturday, Sunday, and holidays the gas levels were: 102–103% TDG, 5.6–5.7 ppm DO, 115–117% nitrogen saturation.

The prednisolone/heat stress was administered according to the following procedure (Jack Frimeth, personal communication, University of Guelph, Guelph, Ontario).

HEAT/STRESS TEST

(April 7, 1987)

1. Inoculate 60 fish intramuscularly with 20 mg/kg of prednisolone acetate.
2. Acclimate the fish to 18 °C over a period of 2 to 4 days.
3. Run the test for 14 days. Examine all fish that die, and all remaining fish at end of test period, for pathogens.

This test is used by Canadian fish health workers to determine if a lot of fish is free of furunculosis prior to moving to a disease-free hatchery. It can probably be expected to cause many other pathogens to show if present.

RESULTS

Mortalities throughout the study were very low. From March 19 through May 11, 1987, the losses were as follows:

	Initial number of fish	Chinook losses	Initial number of fish	Brook trout losses
Controls	2,000	1	400	4
High nitrogen	2,000	4	400	4
Low DO	2,000	6	400	9

Of the prednisolone acetate injected fish, one died the day of injection and one jumped out of the tank the first night. No other mortalities occurred.

Fourteen days after injection, all injected fish were sacrificed and examined for bacterial infections in the following manner. External signs were noted, abdomens were slit and internal signs were noted. BHI agar was streaked with homogenates from kidney and gut of each fish. Gram smears were made from each kidney and FAT spots were prepared from each kidney for ERM with Leetown anti-*Yersinia ruckeri* conjugate #FITC-IGG Lot 1. No signs of infection were seen on any fish. No bacteria were seen in gram stained smears of kidneys. BHI cultures from kidneys were negative for all but one fish which had two colonies of non-pathogens (presumably contaminants). Twenty-four of 67 gut smears were negative and of the remaining 43, 11 yielded *Aeromonas hydrophila*, 2 *Pseudomonas* sp., and the remaining 30 were either not bacteria or non-pathogens. FAT of the 67 kidney smears were negative for ERM.

In addition to this study, production chinook fingerlings were extensively monitored in 1986 and 1987 at all three stations where they were reared. Not a single isolate of ERM was found.

The numbers of chinook examined were as follows:

Hatchery	Year	
	1986	1987
Wolf Lake	117	48
Platte River	20	60
Thompson	20	114

On April 16 the water supply to the high nitrogen tanks was lost, and approximately 60% of the fish died overnight. This was *not* due to disease, but 20 of the dead fish were sampled with FAT anti-BKD. No reactive cells were found. Culture media (BHI) was inoculated from the kidney, liver, and gut of each of the same 20 fish. *Aeromonas hydrophila*, *Pseudomonas* sp., and various non-pathogens were isolated. ERM was not detected.

DISCUSSION

To date ERM has not been isolated in any chinook, adult or production fish, in Michigan. It has not been isolated from any production fish of any species at any State of Michigan fish hatchery, nor has it been isolated from any adult spawners (coho, chinook, or steelhead) from Michigan. It was isolated from a single spawning Skamania steelhead from Lake Michigan at Trails Creek, Indiana, in the spring of 1987, and also a moribund feral brown trout from Lake Michigan near Grand Haven in the spring of 1988.

However, the organism is in the basin and has been isolated from two hatcheries in Illinois and one U. S. Fish and Wildlife Service hatchery in Michigan, as well as from fish in New York and Wisconsin.

Considerable inspection work on Michigan hatchery and spawning run fish provided no isolates that were cytochrome oxidase negative, gram negative rods (which is characteristic of ERM). We have used selective media (Shotts-Waltman) as well as enrichment media (BHI agar) and have attempted isolations from kidney, spleen, and gut. We have not yet had a mortality due to any bacterial septicaemia in chinook, nor have we had any mortality in any cultured salmonid from which ERM has been isolated.

CONCLUSIONS

To this date ERM has not been isolated in chinook from the Little Manistee spawning population nor from a State of Michigan hatchery. Attempts in this study to induce disease by stress and via prednisolone acetate injections did not result in disease or the isolation of ERM or BKD. The origin of ERM in State of Illinois hatcheries is still unknown.

Report approved by W. C. Latta

Typed by G. M. Zurek