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## Prevalence of Larval *Anisakis simplex* in Pen-reared and Wild-caught Salmon (Salmonidae) from Puget Sound, Washington

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**ABSTRACT:** The abundance of parasites of public health significance in pen-reared salmon and wild-caught salmon was compared. Two hundred eighty-seven salmon from Puget Sound, Washington, were examined for third-stage larvae of *Anisakis simplex*. Of these fish, 237 Atlantic salmon (*Salmo salar*), coho salmon (*Oncorhynchus kisutch*) and chinook salmon (*O. tshawytscha*) were reared in commercial salmon pens and 50 sockeye salmon (*O. nerka*) were caught during their spawning migration. All wild-caught salmon were found to be infected with larval *A. simplex*; conversely, all pen-reared fishes lacked such infections. Edible musculature of wild salmon were infected with 581 (87%) nematode larvae. Of other salmon parasites known to infect humans, one *Diphyllobothrium* sp. plerocercoid was collected from each of three of the 50 wild-caught salmon. The study showed that farmed salmon may increase the margin of safety for consumers of raw seafood.

**Key words:** *Anisakis simplex* larvae, prevalence, intensity, parasites, salmon, public health, pen-reared, aquaculture, Puget Sound, *Diphyllobothrium* sp. plerocercoid, *Oncorhynchus* spp., *Salmo salar*.

A public health concern in the United States involves the presence of the third-stage larvae (L<sub>3</sub>) of some ascaridoid nematodes (*Anisakis simplex* and *Pseudoterranova decipiens*) in seafood products. These larval nematodes, when ingested in raw or undercooked fishes, can cause anisakiasis (Bier et al., 1987). In the United States salmon (*Oncorhynchus* spp.) are most commonly implicated in the transmission of this zoonosis.

The general life cycle of anisakid nematodes involves crustaceans (e.g., krill) as transport hosts, fishes (e.g., salmon, rockfishes, cod) as intermediate hosts, and marine mammals (e.g., seals, sea lions, dolphins, whales) as definitive hosts. The prevention of parasitic infections in salmon by interrupting the anisakid nema-

tode's three-host life cycle would be advantageous to consumers. For a variety of reasons such as the impractical task of controlling the diets of the pelagic and anadromous Pacific salmon and the legislative protection of marine mammals, attempts to break the life cycle have been largely abandoned. Research concerning prevention of anisakiasis, therefore, has focused principally on killing the parasites in the edible tissues of commercially important finfish (Gustafson, 1953; Ronald, 1960; Oishi and Hiraoki, 1971; Hauck, 1977; Deardorff, 1988; Deardorff and Throm, 1988).

With the recent advances in aquaculture technology, salmon farming has become a viable and rapidly developing industry. The rearing of salmon in seawater net-pens for the commercial market has been widely used in Europe and quickly gaining acceptance in North America. Because the diet of farmed fishes consists primarily of commercially prepared foods, these fishes may have less opportunity to become infected with anisakid nematodes and other helminths. Thus, the numbers of larval helminths, especially L<sub>3</sub> of *A. simplex*, in pen-reared salmon should be greatly reduced or eliminated. This hypothesis was tested by comparing the numbers of parasites in 237 market-ready, pen-reared salmon with those of 50 wild-caught salmon.

Salmon were collected in the Puget Sound (Washington, USA; 47°30'N, 122°30'W) from June through October 1987. Fifty free-ranging sockeye salmon (*Oncorhynchus nerka*) caught during their migratory spawning run and 237 pen-reared Pacific (*Oncorhynchus* spp.) and

Atlantic (*Salmo salar*) salmon were examined for parasites. Pen-reared salmon were hatchery-reared from eggs, and at smolting, the fingerlings were introduced to seawater pens. From hatchery to harvest, these salmon were fed only commercially processed foods.

Two pen-rearing facilities provided the salmon. The first facility, Farm A (Bremerton, Washington), specializes in rearing only "pan-sized" coho (*Oncorhynchus kisutch*). The coho are harvested approximately 1 yr after stocking. These fishes closely resembled a freshwater trout in size, with a mean ( $\pm$  standard error) length and weight of  $29 \pm 1.8$  cm and  $0.75 \pm 0.2$  kg, respectively. The second facility, Farm B (Manchester, Washington), is an experimental farm that provides fishes held in pens for approximately 2 yr. Atlantic salmon and chinook salmon (*O. tshawytscha*) collected at Farm B weighed 3 to 7 kg ( $7.8 \pm 1.2$ ).

Fishes were transported on ice to the laboratory at Battelle Marine Research Laboratory-Northwest (Sequim, Washington 98382, USA) and remained in a cooler on ice until examination. The heart and the posterior  $\frac{1}{3}$  of the kidney of each fish were examined microscopically for the metacercariae of *Nanophyetes salmincola*. The viscera and musculature of each salmon were individually digested by the pepsin-hydrochloric acid process developed by Stern et al. (1958) as modified by Deardorff and Throm (1988) and digested materials were examined for helminths.

All wild-caught sockeye were infected with *A. simplex* L<sub>3</sub>; 669 L<sub>3</sub> were recovered. Of these anisakid L<sub>3</sub>, 87% were recovered from the edible musculature. Wild salmon were infected with a mean of  $14 \pm 1.2$  nematodes. The range of infection with L<sub>3</sub> in the muscle and viscera of these fish was 0 to 30 and 0 to 15, respectively. One plerocercoid of *Diphyllbothrium* sp. was collected from each of three of the 50 wild-caught fish. In contrast, larval anisakids or plerocercoids were not recovered from 200 coho, 31 chinook or 6 Atlantic salmon from

net-pens. Wild and pen-reared fishes were not infected with *P. decipiens* L<sub>3</sub> or metacercariae of *N. salmincola*.

Anisakiasis, diphyllbothriasis and nanophyetiasis may be acquired by humans if living larval stages are ingested. Anisakiasis is usually caused by the L<sub>3</sub> of *A. simplex*, the "herring worm." This infection may elicit acute, sporadic epigastric distress, often associated with nausea and vomiting which usually occurs within 1 to 12 hr after the infected fish are ingested. At least 50 cases of anisakiasis have occurred in the United States in the past 30 yr; the majority has been reported from areas adjacent to the Pacific Ocean in the past 8 yr (Deardorff et al., 1987).

The pen-reared salmon in Farms A and B were not infected with L<sub>3</sub> of *Anisakis simplex*. Based on our limited study we conclude that fish maintained in nets and fed commercial diets probably will not be infected with these L<sub>3</sub>. Salmon farming, therefore, appears to provide an effective means of interrupting the anisakid nematode life cycle.

Whether or not all farmed salmon will be free of ascaridoid larvae remains uncertain. Some farmers use fresh herring (*Clupea harengus*) to supplement the diets of their fishes. However, the use of these herring for food increases the potential for parasite infection in the pen-reared salmon. In addition, small herring swim in and out of the pens and a variety of marine invertebrates colonize the surface of nets. The gastrointestinal tract of fishes examined in this study contained only commercial feed; however, other pen-reared salmon from Puget Sound examined during the summer months have contained numerous crustaceans and occasionally herring. Therefore, under certain conditions, pen-reared salmon may become exposed to the larval nematodes.

In contrast to pen-reared fishes, 100% of the wild-caught sockeye salmon were infected with *A. simplex* L<sub>3</sub>, of which 87% were encapsulated in the edible musculature. The same prevalence (100%) and

similar intensities in the musculature (75%) were reported by Deardorff and Throm (1988) for sockeye salmon from the Puget Sound area. The prevalence of *A. simplex* L<sub>3</sub> in other species of salmon also is high. Myers (1979) reported that 100% of chum (*Oncorhynchus keta*) and 36% of coho were infected with L<sub>3</sub> and a survey in 1982 (J. W. Bier, pers. comm.) found 98% of coho, 97% of pink (*O. gorbuscha*), 79% of chinook and 100% of sockeye were infected with *A. simplex* L<sub>3</sub>.

*Diphyllbothrium* spp. plerocercoids and *N. salmincola* metacercariae were not found in pen-reared fishes, apparently because the fishes were raised in well water during the freshwater phase of development. Without exposure to freshwater rivers and lakes in areas where the transmission of these parasites normally occurs, these salmon do not become infected.

Diphyllobothriasis is caused by the plerocercoid of *Diphyllbothrium* spp. Signs and symptoms in humans are abdominal cramps, diarrhea, nausea, fatigue, weight loss, and sometimes vitamin B<sub>12</sub> deficiency with onset approximately 10 days after consumption of raw or undercooked fish. According to Bylund (1982), de Carneri and Vita (1973) estimated the number of human cases of diphyllobothriasis in America is <100,000. Salmon are suspected as the agent of transmission in some of the cases.

Nanophyetiasis, often called "fish flu," is transmitted by salmonids and is caused by the digenean *N. salmincola*. Eastburn et al. (1987) conclusively demonstrated that humans could be infected with this parasite. They reported that 10 individuals from the Pacific northwestern coast experienced abdominal discomfort, diarrhea, fatigue, flatulence, nausea, vomiting and weight loss; others showed either no symptoms or very mild clinical symptoms. The number of human cases in the United States has increased to >20 within 1 yr of the original report (T. R. Fritsche, pers. comm.). Niclosamide and bithionol are effective anthelmintic treatments for nanophyetiasis

(Eastburn et al., 1987; T. R. Fritsche, pers. comm.) and niclosamide is a proven treatment for diphyllobothriasis. There is no anthelmintic treatment for anisakiasis.

Growth of the salmon farming industry worldwide is expected to triple from 1985 to 1999, and conservative worldwide projections for salmon production are estimated at 165,000 metric tons by 1990 according to industry reports (Fitzgerald, 1987). Problems such as infectious diseases (Kent and Elston, 1987), waterborne toxicants (Kent et al., 1988), difficulties in obtaining permits, nonavailability of good sites for fish pens, environmental and aesthetic impact of the salmon farms, and competitive pricing with wild-caught salmonids represent major hurdles to the projected growth of the industry in the United States. Nevertheless, the salmon farming industry appears to have successfully confronted these problems and the public has begun to see both domestic and foreign pen-reared salmon in markets and restaurants.

The risk of parasitic infection from wild-caught salmon to U.S. consumers remains slight because temperature extremes can kill encapsulated L<sub>3</sub> of *A. simplex* and, presumably, other parasites. Thorough cooking or the sufficiently cold temperatures of domestic or commercial freezers are effective in killing parasites in whole salmon. A U.S. Food and Drug Administration Code Interpretation released in 1987 stated that "Fishery products which are not cooked throughout to 140 F (60 C) or above, must have been or must, before service or sale in ready-to-eat form, be blast frozen to -31 F (-35 C) or below for 15 hours or regularly frozen to -10 F (-23 C) or below for 168 hours (7 days)." These practices, unfortunately, are not always followed. As a result, the numbers of new case reports of anisakiasis and nanophyetiasis continue to increase. The incidence of diphyllobothriasis may also be increasing, although the exact number of cases in the United States is unknown. Close monitoring of human infections by the Centers

for Disease Control (1981) showed that the incidence of fish tapeworm disease in California, Oregon, Washington, Alaska and Hawaii increased nearly four-fold, from 17 in 1979 to 59 in 1980. The current trend of Americans to sample various ethnic and cosmopolitan foods that may involve raw or undercooked seafoods and to cook seafoods for shorter periods of time may increase the incidence of these zoonoses. For those who prefer raw salmon dishes, however, farmed salmon appear to accommodate the tastes of a growing number of consumers of raw salmon products while increasing the margin of safety from parasitic infections.

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