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Loma sp. in Salmonids from the Eastern United States: Associated Lesions in Rainbow Trout

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Abstract.—A microsporidian of the genus *Loma* was noted in the gills of rainbow trout *Oncorhynchus mykiss* from a state hatchery (Buford Trout Hatchery) in Georgia. Mortalities of varying severity occur at this hatchery every fall, and the microsporidian was noted during an experiment from August 1991 to January 1992 to determine the effects of water source on disease. Infections first appeared to be systemic in the October sample; xenomas were observed in heart, spleen, and peripheral vessel walls. The presence of unidentified intracellular material preceded the appearance of xenomas in all tissues, but whether this material was associated with inflammation or represented immature stages of the parasite has yet to be determined. These structures were also noted in the intestine and liver, although xenomas were not noted in these organs. Mature xenomas did not elicit an inflammatory response but appeared to be short-lived. When the xenoma wall ruptured and released spores, an inflammatory response was again observed. The prevalence and severity of the infection were determined in fish maintained in troughs with well water, Chattahoochee River water, or hatchery (treated river) water. The infection tended to be more prevalent and more severe in fish maintained in the hatchery or river water than in those maintained in the well water. Stress induced by poor water quality may increase mortality from this parasite. This report extends the range of *Loma* sp. into the eastern United States.

Microsporidians of the genus *Loma* have been associated with disease in salmonids primarily on the west coast of the United States and Canada. Mortalities of *Loma*-infected rainbow trout *Oncorhynchus mykiss*, steelhead (anadromous *O. mykiss*), and kokanee *O. nerka* in California and British Columbia were first reported by Wales and Wolf (1955). The microsporidian was observed in gills of hatchery and wild salmonids, as well as in wild sculpin *Cottus* sp. The organism was first named *Plistophora salmonae* by Putz et al. (1965). Morrison and Sprague (1981a) described a *Loma* sp. in brook trout *Salvelinus fontinalis* from Nova Scotia and suggested that all microsporidia reported in the gills of salmonids belong to the genus *Loma*. These authors recognized that all *Loma* spp. described to date may have represented one species and that more information was needed to accurately identify these parasites at the species level. In 1983, two species, *L. salmonae* and *L. fontinalis*, were recognized and compared through electron microscopy studies. These species differ primarily in spore size, number of turns of the polar filament, and vacuolization of the sporoblast (Morrison and Sprague 1983).

Although *Loma* spp. are considered parasites of the gill, systemic infections have been reported in chinook salmon *O. tshawytscha* from Alaska (Hauck 1984) and in seawater-reared coho salmon *O. kisutch* from the state of Washington (Kent et al. 1989). *Loma salmonae* infects endothelial cells

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and has been observed in the vasculature of various organs (Hauck 1984; Kent et al. 1989).

Losses of trout, often major, have occurred annually at Buford Trout Hatchery since fall 1976. Jones, Edmunds and Associates (1982) have attributed these fish losses to high levels of iron (Fe) and manganese (Mn) in the water and suggested that increasing water hardness would effectively prevent mortality. However, further studies have not supported this finding (Couch 1990). Because iron is known to be important in susceptibility to disease (Weinberg 1978), we hypothesized that iron may be interacting with an infectious agent to cause the mortalities. The fish is this study were part of an experiment designed to evaluate effects of water source on mortality and tissue damage. During the course of the experiment, a microsporidian (*Loma* sp.) was noted in the gills of rainbow trout. Since that time, a similar microsporidian has been observed in tissues from brook trout and brown trout *Salmo trutta* at the same hatchery, as well as in rainbow trout reared at state hatcheries in West Virginia and Pennsylvania, and rainbow trout and coho salmon from a private producer in Virginia. The progression of lesions thought to be associated with this parasite and the influences of water quality are presented in this report.

Methods

Buford Trout Hatchery is located on the Chattahoochee River, approximately 3 km downstream from Buford Dam, and is supplied with water continuously pumped from the river. For our experiment, duplicate troughs (90 L) were set up to receive well water, river water, or hatchery water. Hatchery water was river water subjected to any treatments that the hatchery proper received, such as recirculation and chemical treatments. The river water tanks received water drawn directly from the Chattahoochee River. Well water was pumped from a ground well about 10 m deep. One hundred ten Winthrop strain rainbow trout (average total length, 13 cm) were stocked from the hatchery into each trough. This level of stocking approximated the density index of 0.25 (English units) routinely maintained at Buford hatchery. (Density index equals the total weight of fish in pounds divided by the product of raceway volume in cubic feet and fish length in inches: Piper et al. 1982). The experimental fish had been reared from eggs at Buford hatchery and received the same feed as fish in the hatchery proper. Daily feeding rates and mortalities were recorded for each trough.

From August 1991 through January 1992 (except December), 5 fish were randomly sampled once every 2 weeks from each trough (10 fish for each water source). Fish were anesthetized in neutral-buffered tricaine methanesulfonate (MS-222) and bled from the caudal vein. Fish were then necropsied and pieces of gill, heart, head kidney, trunk kidney, intestine, stomach, pyloric ceca, liver, and spleen were fixed in 10% neutral-buffered formalin. Dead fish were counted and removed from the troughs daily. These fish were fixed whole and examined.

Water samples were collected twice a week from August through December. A handheld YSI dissolved oxygen (DO) meter was used to measure temperature and DO. Hach chemical kits were used to measure total hardness, pH, and total Fe and total Mn concentrations. A handheld Weiss saturometer was used to measure percent gas saturation.

Fixed tissue was embedded in paraffin and cut 3 μm thick. Sections were stained with hematoxylin and eosin (H&E). Special stains included Perl's Prussian blue for iron, periodic acid–Schiff, and iron hematoxylin (Luna 1968).

For transmission electron microscopy, tissues were fixed in cold (4°C) 2% (volume/volume) glutaraldehyde in a 0.27 M sodium cacodylate buffer, processed, and embedded in Dow epoxy resin (DER) 732 (Kocan et al. 1980). Thick sections (1.5 μm) stained with Mallory's stain (Richardson et al. 1960) were examined by light microscopy. Ultrathin sections (silver-reflective) were cut with a Sorvall MT-5000 ultramicrotome and a Diatome diamond knife. These sections were stained with uranyl acetate and lead citrate (Venable and Coggeshall 1965) and observed and photographed in a JEOL CX 100 electron microscope operated at 80 kV. Measurements of mature spores were made by examination of 20 spores in thick sections under a light microscope. In addition, 25 fresh spores were measured in unstained wet mounts of a ruptured gill xenoma.

To study the progression of the disease and compare the response of infected fish in the different water systems, lesions were classified according to a modified version of the method described by Reimschuessel et al. (1992). Each lesion was given a classification code based on location, type of response (such as inflammation or necrosis), and extent of response (focal, multifocal, or diffuse). Each tissue section was then given a numerical rating based on a five-point system for severity of the lesion (amount of tissue involved):

(1) minimal, (2) mild, (3) moderate, (4) marked, and (5) severe.

Results

Parasite Identification

Identification of these microsporidians was based on size of the mature spores, turns of the polar filament, and presence of xenomas in the gills of salmonid fishes (Kent et al. 1989). Mean (±SD) dimensions of the mature spores were 3.6 (±0.1) by 1.7 (±0.1) μm, as determined by measuring 20 spores in thick sections. Fresh spores released from gill xenomas were somewhat larger: 4.8 (±0.4) by 2.6 (±0.3) μm.

Electron Microscopy

Xenomas from rainbow trout gills contained several stages (Figure 1), including meronts, sporoblasts, and mature spores. The polar filament passed diagonally through the anterior third of the spore and 14–17 turns of the polar filament could be observed in electron micrographs. Typical laminate and vesiculate parts of the polarplast were evident (Figure 2).

Histopathology

The most numerous and prominent lesions associated with the microsporidian were found in gills. The most common lesions were (1) multifocal areas of chronic inflammation in the gill filament, (2) xenomas, (3) multifocal areas of granulation or fibrous tissue in the gill filament, and (4) multifocal areas of lamellar fusion. Mature xenomas were most often observed in lamellae, just under the epithelium and in close association with pillar cells (Figure 3A). Xenomas were less frequently observed within the vasculature of filaments (Figure 3B). As the wall surrounding the xenoma lost its integrity, inflammatory cells, primarily macrophages, began to infiltrate the xenoma (Figure 3C, D). Other lesions observed in the gill were focal areas of lamellar fusion and focal areas of inflammation and necrosis in filaments (Figure 3A). Often spores were observed in these areas. The fusion of lamellae was primarily due to inflammatory cells and fibrosis. Xenomas were also occasionally found attached to vessel walls in the head region of some fish that died during the experiment (Figure 4A).

The heart was the second most frequent location of lesions after the gills, and here xenomas were most commonly seen in the ventricular endocardium (Figure 4B). In addition, perivascular edema and inflammation were noted around coronary arteries in the visceral epicardium. Individual spores were observed in this layer (Figure 4C).

In the spleen, xenomas were associated with endothelium of capillaries (Figure 5A). Perivascular inflammation was observed in the ellipsoid tissue surrounding splenic blood vessels. Splenic white pulp showed a proliferation of activated (vacuolated) macrophages. Occasionally, focal areas of granulomatous inflammation and necrosis were observed in splenic tissue (Figure 5B). Within these areas individual spores could be found (Figure 5C).

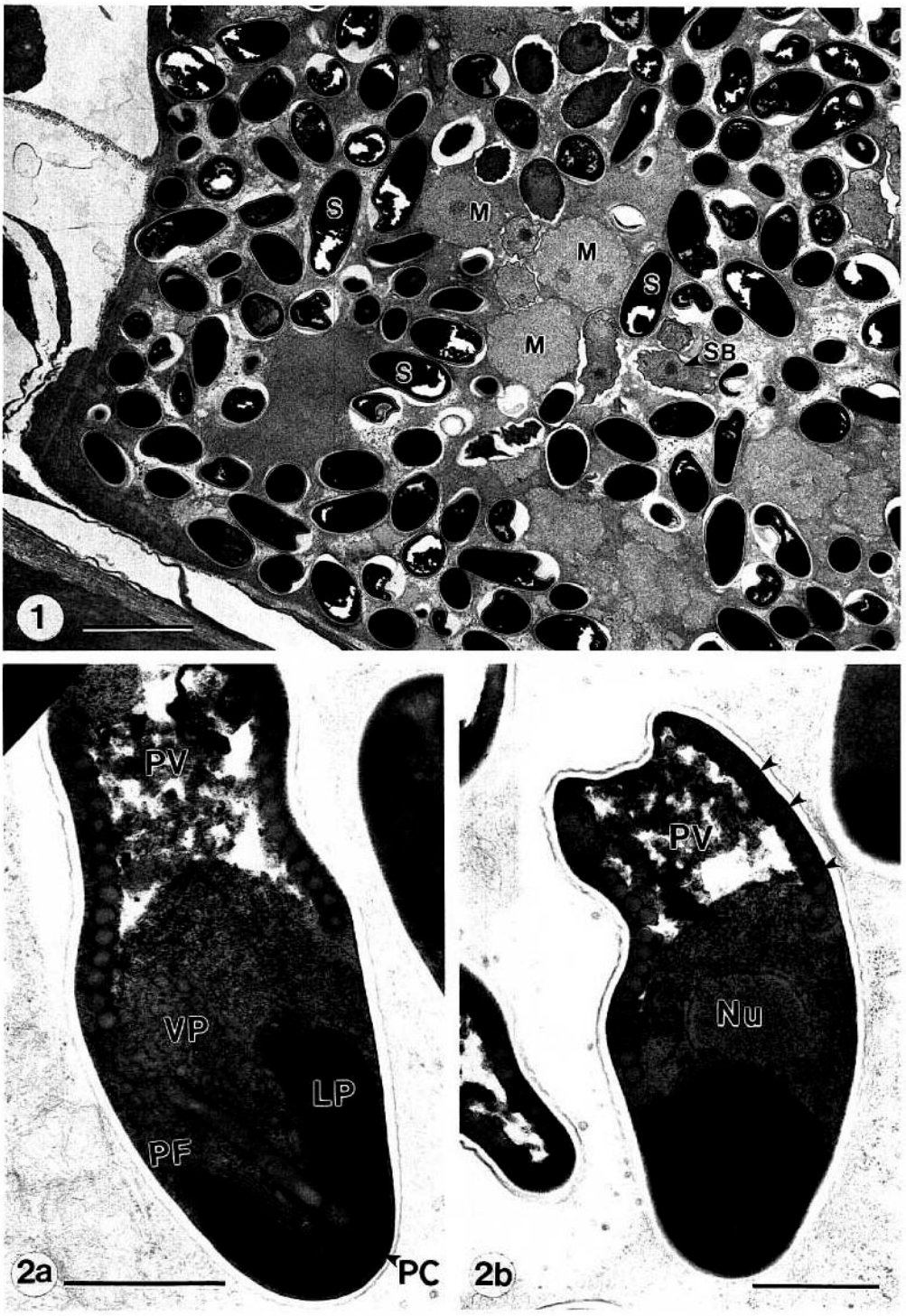
Xenomas were not observed in the liver in rainbow trout; however, multifocal areas of perivascular and periductal inflammation were observed. These areas were located primarily around the hepatic arteries and progressed in some cases to extensive chronic inflammation composed of macrophages, lymphocytes, and fibrosis. Occasionally, necrotic areas of the vessel walls were evident, and they appeared to result in the accumulation of inflammatory cells and thrombocytes attaching to the wall. In addition, multifocal areas of chronic inflammation were found in the liver parenchyma.

Xenomas were not observed in the intestine of rainbow trout either. We did note many individual necrotic cells, chronic inflammation in the submucosa, and the presence of unidentified intracellular structures.

Within xenomas, mature spores as well as various life stages of the microsporidian could be seen (Figure 1). Not only individual spores but also unidentified intracellular structures, often with a budding or morula appearance, were observed in phagocytes of the heart (Figure 4C), spleen (Figure 5D), liver, kidney, intestine, and gills. Usually seen in inflamed areas, they resembled structures observed within xenomas (Figure 5A).

Progression and Prevalence of the Infection in Relation to Water Source

The progression of lesions believed to be associated with the infection was followed in fish maintained in the three water systems, and the histological classification of Reimschuessel et al. (1992) was used to record observations. A number of trends were detected (Table 1). In August, fish from all groups had small numbers of the unidentified intracellular structures in the gill but no xenomas were observed. Inflammation and some lamellar fusion occurred in gill tissue of all fish. By September, xenomas were observed in gills of fish from the well water and hatchery water troughs,



FIGURES 1, 2.—Electron micrographs of *Loma* sp. infection. **Figure 1.** Xenoma in a rainbow trout gill, showing meronts (M), sporoblasts (SB), and spores (S). Bar = 5 μm. **Figure 2.** *Loma* sp. spores showing **(a)** posterior vacuole (PV), vesicular polaroplast (VP), lamellar polaroplast (LP), polar filament (PF), polar cap (PC), and **(b)** nucleus (Nu) and polar filament in cross section (arrows). Bars = 1 μm.

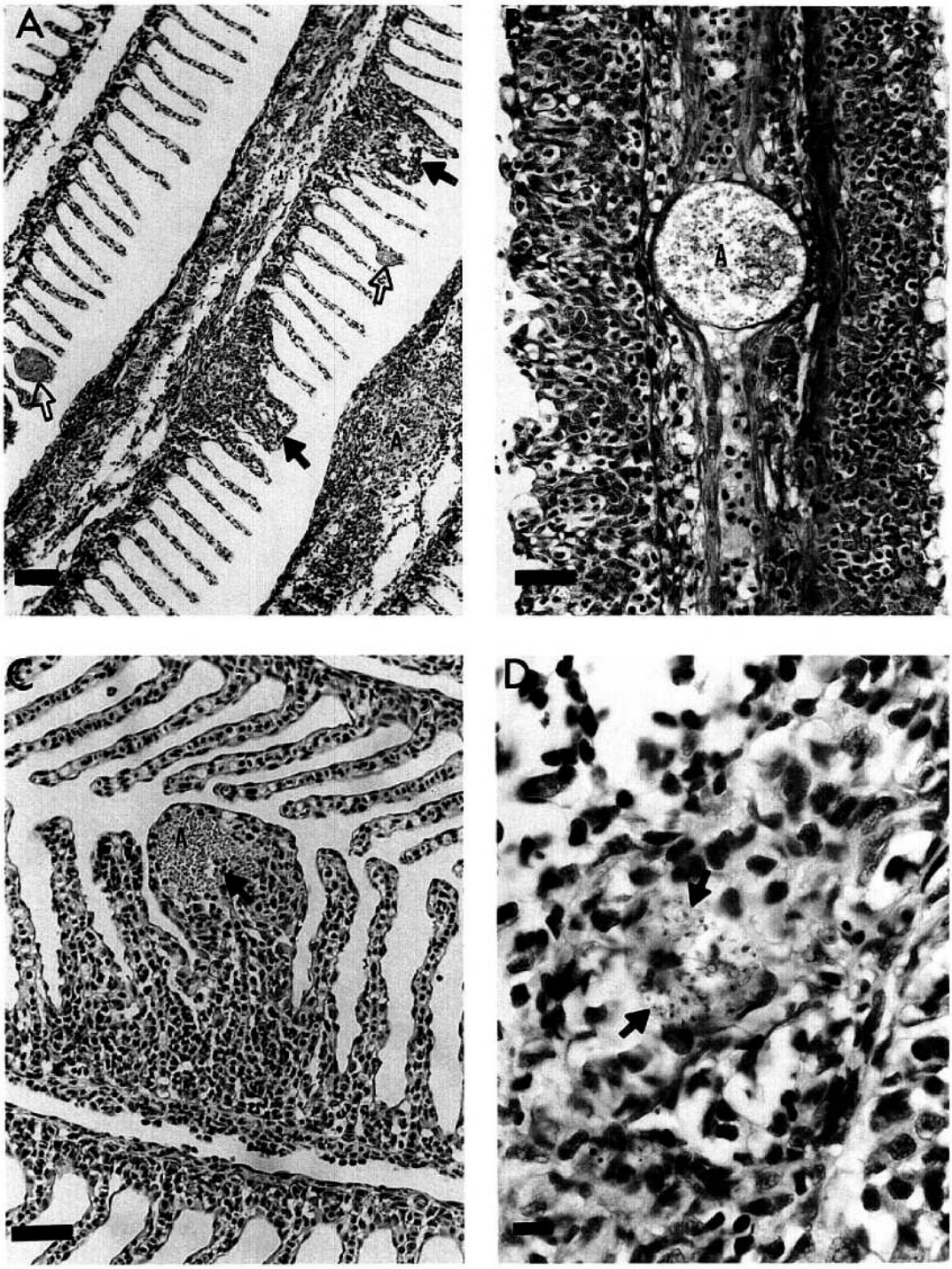


FIGURE 3.—Lesions noted in gill tissue of rainbow trout infected with *Loma* sp. (H&E stain): (A) mature xenomas (open arrows), and areas of necrosis and inflammation within the filament (small A) and between lamellae (closed arrow; bar = 100 μm); (B) xenoma (A) within a vessel of the filament (bar = 40 μm); (C) inflammatory cells (arrow) infiltrating a xenoma (A; bar = 50 μm); (D) higher magnification of an area of chronic inflammation within gill filament, containing spores (arrows; bar = 10 μm).

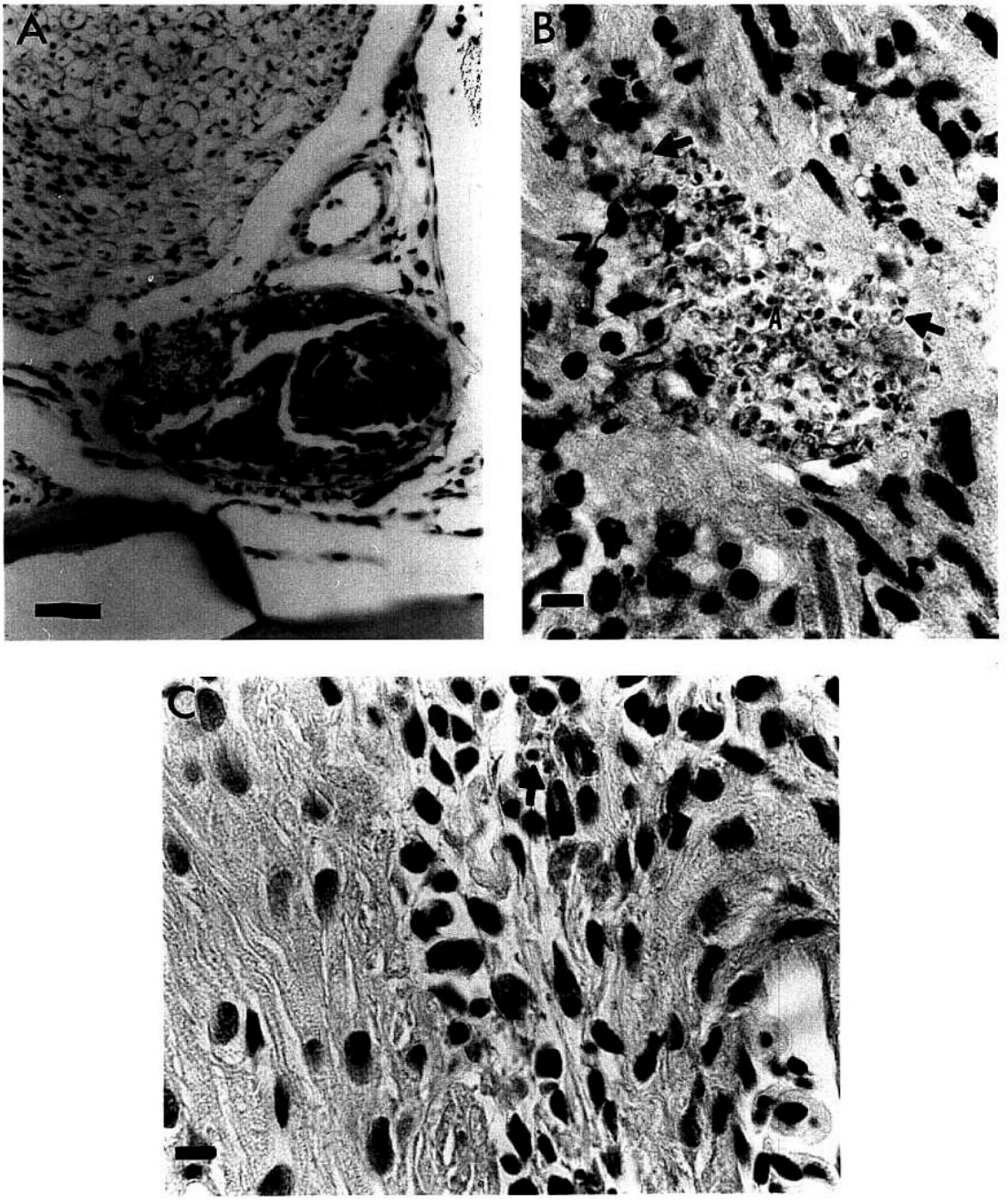


FIGURE 4.—Lesions of the vasculature and heart of rainbow trout infected with *Loma* sp. (H&E stain): (A) xenoma (arrow) attached to endothelium of a vessel wall, in the head region, posterior and dorsal to the inner ear (bar = 40 μm); (B) xenoma with wall no longer intact (A), and inflammatory cells and free spores (arrows) in the heart (bar = 10 μm); (C) chronic inflammation and spores (arrow), and unidentified intracellular structures with budding appearance, in the epicardium (bar = 10 μm).

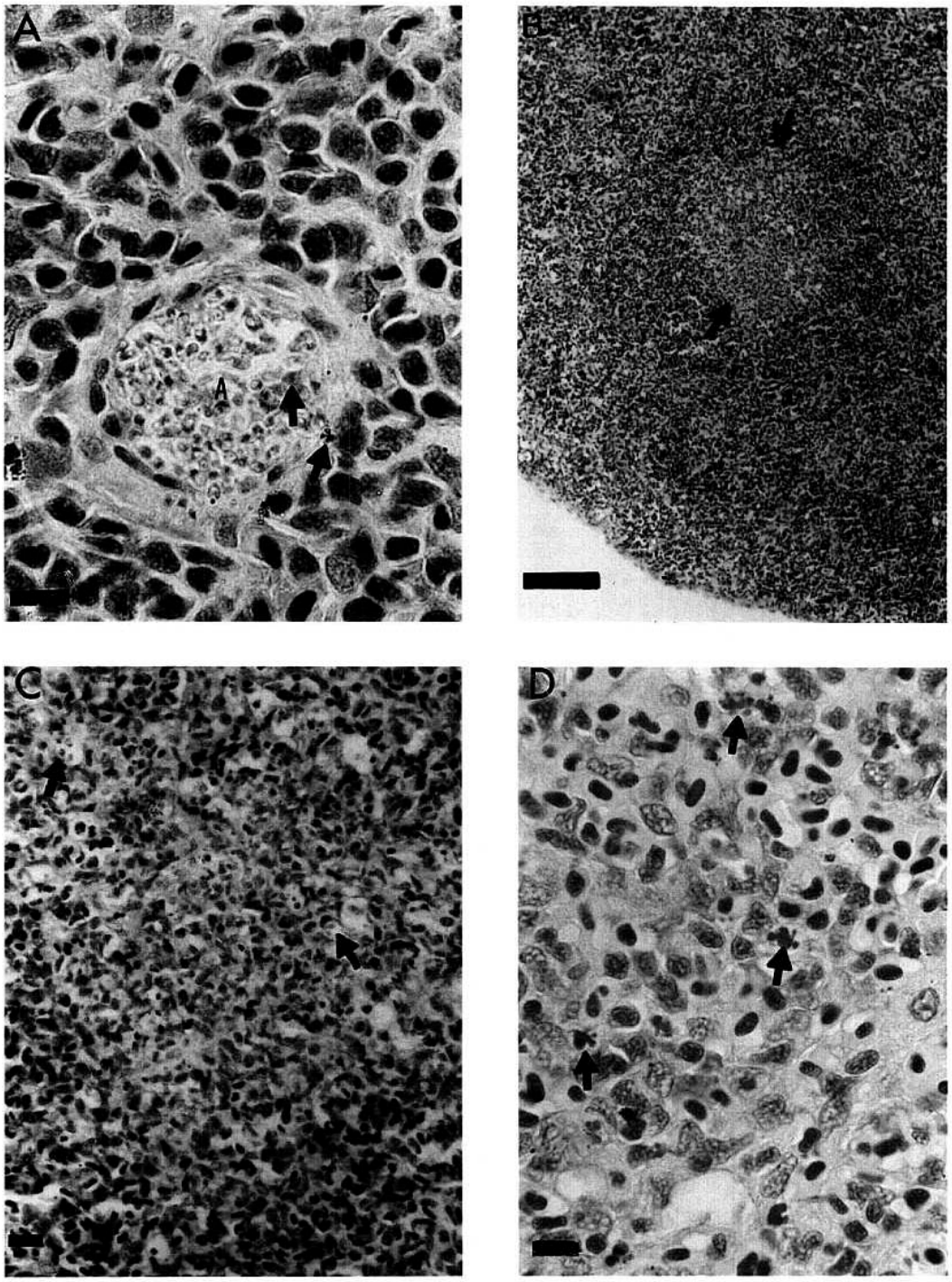


FIGURE 5.—Lesions in the spleen of rainbow trout infected with *Loma* sp. (H&E stain): **(A)** xenoma (small A) containing meronts or sporoblasts (arrows; bar = 10 μm); **(B)** focal area of chronic inflammation and necrosis (arrow; bar = 100 μm); **(C)** higher magnification of the area of inflammation and necrosis, showing individual, intracellular spores (arrows; bar = 10 μm); **(D)** splenic tissue with intracellular structures (arrows; bar = 10 μm).

and intracellular structures were noted in all groups. In October, intracellular structures and xenomas were observed in gills of fish from all water sources. At this time, the severity was greatest in fish from the hatchery water, intermediate in fish from river water troughs, and lowest in those from the well water troughs. Also, intracellular structures or xenomas appeared for the first time in hearts of fish from the hatchery and river water groups. November was the month of peak prevalence for gill xenomas in all groups, but the prevalence was lowest in fish from the well water, intermediate in those from the hatchery water, and highest in those from the river water. By January there were no xenomas or intracellular structures in gills of fish maintained in well water. The parasite was not noted at any time in the hearts of fish maintained in well water. Fish held in river water had intracellular structures in the gill but none in the heart, whereas fish held in hatchery water had xenomas and intracellular structures in gill and heart.

Intracellular structures were commonly observed in the phagocytes of the spleen. Xenomas were only rarely seen at this location and throughout the course of the experiment were noted in splenic tissue of only one fish from each of the water sources.

TABLE 1.—Prevalence and severity of lesions observed in the gill and heart of rainbow trout from the Buford (Georgia) hatchery infected with *Loma* sp. Prevalence in each sample ($N = 9$ or 10) is presented as percentage followed in parentheses by the mean severity score for those fish having the lesion. Scores were based on a rating of 1–5 (minimal to severe lesions).

		Gill		Heart	
Month	Water supply	Xenomas	Intracellular structures	Xenomas	Intracellular structures
Aug	Well	0	89 (1.1)	0	0
	Hatchery	0	100 (1.3)	0	0
	River	0	44 (1.0)	0	0
Sep	Well	20 (1.0)	100 (1.2)	0	0
	Hatchery	10 (1.0)	80 (1.1)	0	0
	River	0	80 (1.3)	0	0
Oct	Well	11 (1.0)	67 (1.0)	0	0
	Hatchery	44 (1.8)	67 (2.8)	0	22 (1.0)
	River	22 (1.0)	67 (1.7)	20 (1.0)	0
Nov	Well	44 (1.5)	67 (1.8)	0	0
	Hatchery	70 (1.5)	80 (1.8)	0	0
	River	78 (1.5)	89 (1.6)	0	0
Jan	Well	0	0	0	0
	Hatchery	43 (4.3)	43 (3.7)	43 (2.3)	0
	River	0	50 (1.0)	0	0

Water Quality

Water quality in the Chattahoochee River deteriorates during fall and early winter as a result of stratification of Lake Sidney Lanier. Fluctuations in water quality are amplified by hydropower facility water releases from Buford Dam. Temperature, DO, and total Fe + Mn levels showed marked differences between "low flow" (normal river flow) and "high flow" (releases from the dam) in the hatchery and river water troughs (Figure 6). In these troughs, temperatures varied from 10.6 to 15.5°C, DO from 4.8 to 7.0 mg/L, and total Fe + Mn from 0.5 to 3.2 mg/L throughout the experiment. Fluctuations were slightly greater in the hatchery tanks than in the river water tanks. Perhaps more important than the fluctuations over the course of the experiment were the fluctuations between high- and low-flow periods in certain months. For instance, in the river water troughs in October, temperatures fluctuated between 11.8 and 15.6°C in a 12-h period on numerous days.

In the well water troughs, water characteristics remained relatively constant (Figure 6). Temperature was 15 ± 0.5°C, DO was between 7.9 and 6.4 mg/L, and total Fe + Mn concentration was 0.4 mg/L. Other water quality variables—pH, total hardness, and gas supersaturation—showed minimal fluctuation in all water supplies.

Mortality

Moribund fish appeared dark and frequently had eroded opercles. Occasionally moribund fish also showed a loss of equilibrium. Only one fish died in troughs receiving well water during the study period. Eight fish died in troughs with hatchery water and 19 died in troughs receiving river water. All dead fish had xenomas in the gills.

Discussion

The mature spores observed in this study were similar in size to those of *Loma* species previously identified as *L. salmonae* from salmonids. Reported dimensions of spores of *L. salmonae* from rainbow trout are 4.5 × 2.2 μm (Putz et al. 1965) and from coho salmon are 4.4 × 2.3 μm in wet mounts from fixed tissue (Kent et al. 1989) and 4.0 × 2.1 μm in glutaraldehyde-fixed, processed tissue (Magor 1987). Spores reported from brook trout as *L. fontinalis* by Morrison and Sprague (1983) were 3.7 × 2.2 μm and were measured in photographs of thick sections. Further electron microscopy studies to identify this parasite and compare spores from the various outbreaks have

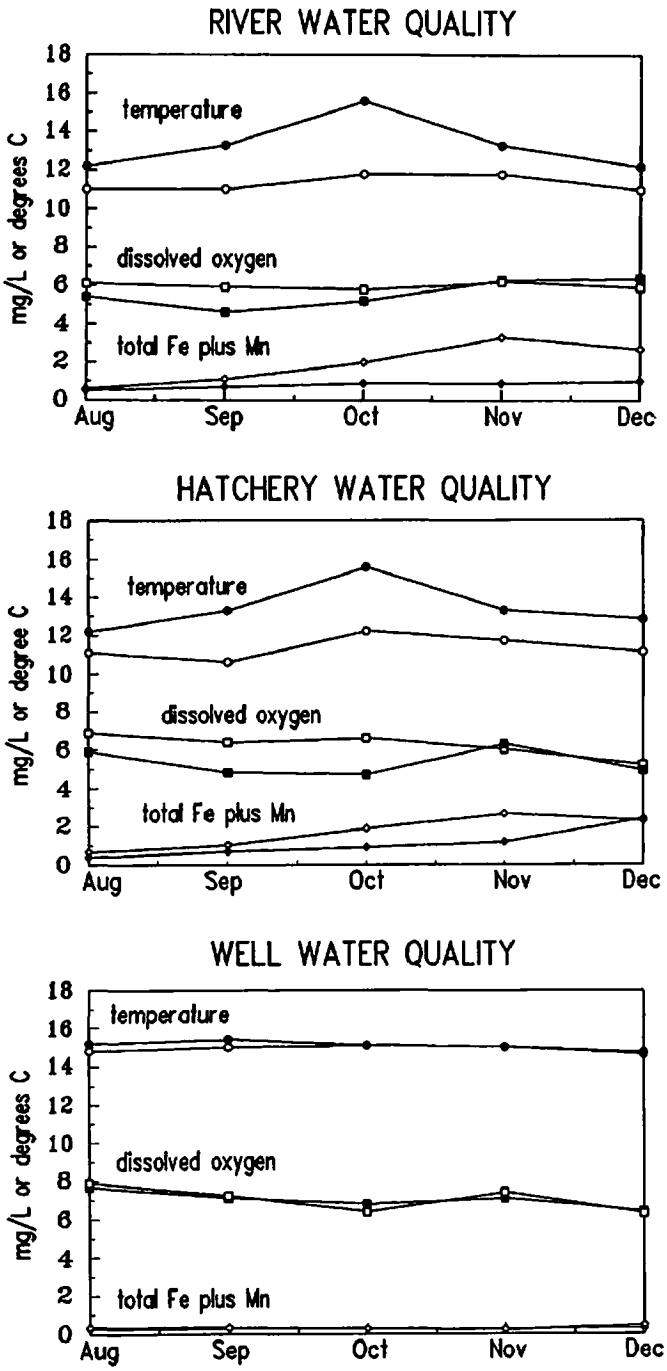


FIGURE 6.—Fluctuations in water quality in troughs supplied with water from the Chattahoochee River, the Buford hatchery (treated river water), or a well. Open symbols indicate mean low flow and closed symbols indicate mean high flow.

been completed (J. A. Bader and colleagues, University of Georgia, unpublished).

Previous reports of *Loma* in salmonids maintained in freshwater have indicated gill tissue as the primary organ affected and have suggested that the microsporidium probably causes disease by occupying a large portion of the gill with xenomas (Wales and Wolf 1955; Magor 1987; Kent et al.

1989). The lesions we noted in the gills were similar to those described for coho salmon (Kent et al. 1989) and for Atlantic cod *Gadus morhua* gills infected with *Loma morhua* (Morrison and Sprague 1981b). Our observations support the hypothesis that when mature cysts lose their integrity, phagocytes invade the spore mass and spores (and possibly immature stages) are released. We believe that the unidentified intracellular structures observed in gill tissue, as well as in other organs, may represent a developmental stage of this parasite. If this is true, the initial infection may occur in the gill (or in the intestine, as described by Loubes et al. 1984) and this stage also elicits an inflammatory response that may rid the tissue of the infection at this stage. If the parasite is not eliminated, it can multiply, form a xenoma, and hence be protected from the inflammatory reaction for a time.

Systemic infections of *Loma* sp. have been reported in chinook salmon juveniles from Alaska (Hauck 1984) and in coho salmon from Washington (Kent et al. 1989). Many of the same lesions reported by the previous investigators were noted in this study. In addition, in the spleen, gill, and heart, inflammation was associated with the unidentified structures as well as with individual spores. Xenomas were found in only one spleen from each treatment group. However, the diffuse areas of chronic inflammation containing spores were most likely caused by the disintegration of xenomas and resulting infiltration of inflammatory cells.

Although xenomas were not observed in the intestine or liver, we did note inflammation and the unidentified structures. We recognize that these lesions may also have been associated with other pathogens or water quality problems. The hatchery has had a history of *Yersinia ruckeri, Aeromonas salmonicida,* and *Flexibacter columnaris,* as well as occasional outbreaks of *Ichthyophthirius.* However, we did not observe gross or histological signs of any of these diseases in our experimental troughs.

Dykova and Lom (1980) suggested that most xenomas are short-lived, and unless spores are discharged to the external environment, they will be destroyed by the host response, primarily phagocytes. Previous investigators have suggested that stressful conditions, which suppress optimal functioning of phagocytes, may result in systemic or severe infections and mortality (Hauck 1984; Magor 1987). Our results lend support to this hypothesis. We believe the fluctuating temperature and DO, as well as the increasing levels of Fe and Mn were factors contributing to the greater prevalence and severity of infections in fish kept in the hatchery and river water. In addition, fish in well water appeared to be able to rid the gills of the infection by January, whereas xenomas were still apparent in fish in the hatchery water.

This report extends the known range of *Loma* sp. to the eastern United States. Brook trout and brown trout are also maintained at the Buford hatchery, and the parasite has been observed in gill tissue of these species. Further electron microscopy studies will be required to determine if the same *Loma* species is infecting all fish species and if this is the same species that has been reported from salmonids on the west coast of North America.

Acknowledgments

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