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Experimental and natural host specificity of *Loma salmonae* (Microsporidia)

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ABSTRACT: The microsporidian *Loma salmonae* (Putz, Hoffman & Dunbar, 1965) Morrison & Sprague, 1981 has caused significant gill disease in Pacific salmon *Oncorhynchus* spp. Host specificity of the parasite was examined experimentally by per os challenge of selected salmonids and non-salmonids with infective chinook salmon *O. tshawytscha* gill material. Pink *Oncorhynchus gorbuscha* and chum salmon *O. keta*, brown *Salmo trutta* and brook trout *Salvelinus fontinalis*, and chinook salmon (controls) were positive, whereas Atlantic salmon *Salmo salar* and Arctic char *Salvelinus alpinus* were negative. In addition, no non-salmonids were susceptible to experimental exposure. Wild Pacific salmon species in British Columbia, Canada, were examined for *L. salmonae* during their freshwater life history stages (smolts, prespawning, spawning). All stages were infected, although infections in smolts were only detectable using a *L. salmonae*-specific PCR test. Many previous *Loma* spp. described from *Oncorhychus* spp. are likely *L. salmonae* based on host, parasite morphology, and site of infection.

KEY WORDS: *Loma salmonae* · Microsporidia · Transmission · Host specificity

INTRODUCTION

Loma salmonae (Putz, Hoffman & Dunbar, 1965) Morrison & Sprague, 1981 is a serious microsporidian pathogen of Pacific salmon *Oncorhynchus* spp. in the Pacific Northwest. The parasite infects endothelial cells, predominately within the gills, forming white cyst-like structures, referred to as xenomas (Shaw et al. 1998, Shaw & Kent 1999).

Loma salmonae infects mainly Pacific salmon species: chinook salmon *Oncorhynchus tshawytscha* (Kent et al. 1995, Shaw & Kent 1999); coho salmon *O. kisutch* (Bekhti & Bouix 1985, Kent et al. 1989); sockeye and kokanee salmon *O. nerka* (Wales & Wolf 1955, Putz et al. 1965); and rainbow trout *O. mykiss* (Wales & Wolf 1955, Putz et al. 1965, Morrison & Sprague 1983, Bruno et al. 1995). However, Poynton (1986) described the parasite in brown trout *Salmo trutta*, and the parasite has been reported in an unidentified *Cottus* sp. (Wales & Wolf 1955, Putz et al. 1965). Bader et al. (1998) described *Loma* cf. *salmonae* in brook trout *Salvelinus fontinalis*, whereas Speare et al. (1998a) was unable to infect brook trout with the parasite.

Several other *Loma* spp. have been found in various marine and freshwater species (Kent et al. 1998); however, as many of these species are morphologically indistinguishable, it is possible that some of these may represent *L. salmonae* in a non-salmonid host. We have addressed this question in 2 studies (Kent et al. 1995, Shaw et al. 1997), which suggested that *L. salmonae* infects only salmonids.

The present study represents a more thorough examination of *Loma salmonae* host specificity using laboratory transmission. We also examined various freshwater life stages (smolts, prespawning, spawning) of Pacific salmon spp. in British Columbia, Canada, and from the Yukon Territory in an attempt to elucidate the natural host range of *L. salmonae* in western Canada.

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MATERIALS AND METHODS

Host susceptibility. Hosts were tested as outlined in Table 1 following the protocol of Kent et al. (1995) and Shaw et al. (1998). Test fish (n = 20) of each species were placed in a tank (fresh or seawater dependent on fish species) with 10 positive control chinook salmon and were not fed for 2 d prior to exposure. Only 10 goldfish *Carassius auratus* and 10 guppies *Poecilia reticulata* were tested. Fish were fed macerated gill tissue infected with *Loma salmonae* over 3 d. During feeding water flow was turned off for 2 h to facilitate ingestion of gill tissue. On the fourth day 10 experimental fish and 5 controls were removed and infected per os using a syringe containing a gill slurry (see Shaw et al. 1998); their fins were clipped, and the fish were placed back in the tank. All fish were examined 56 d later by wet mount and histology for *L. salmonae*, a time that we have previously determined is optimum for the detection of experimental infections (Shaw et al. 1998). After wet mount examination, gills of fish were placed in Davidson's solution (Humason 1979), processed using standard histological techniques, stained with hematoxylin and eosin, and examined at ×100.

When test species were non-salmonids and smaller than controls, the test species were kept in a separate tank during the feeding of macerated gill tissue. On Day 4 all fish were placed in the same tank.

Wild Pacific salmon examination. Salmonids were collected as outlined in Table 2. Fish were killed with a blow to the head or overdosed with MS-222. The first left gill arch was removed and examined by wet mount at ×100 for smolts and ×25 for prespawning and spawning fish. Intensity of infection was measured from the average of 3 counts of the number of xenomas per ×100 field of view (1.5 mm diameter) and then converted to mm^2. A subsample of the first left gill arch was taken for large (spawning) fish. Total length and weight was recorded for smolts only. After examination by wet mount gills of smolts were placed in 95% ethanol and tested using a *Loma salmonae*-specific PCR test as described by Docker et al. (1997).

In addition to samples collected directly from wild fish a total of 140 sockeye salmon smolts were collected at Great Central Lake, Vancouver Island, British Columbia, on 22 March 1997 with 65 examined initially, and 65 held on ambient seawater (9 to 14°C) for 1 yr before being examined for *Loma salmonae* (wet mount only). Smolts examined on 8 May 1998 were sorted into a 1997 year class (≤3.0 g) and fish 2 yr or older (≥8.0 g).

RESULTS

All 5 non-salmonid species that were experimentally exposed did not develop *Loma salmonae* infections,

Table 1. Prevalence of *Loma salmonae* in fishes tested for susceptibility by feeding infected macerated gill tissue and by per os administration of an infected gill slurry. Controls were chinook salmon *Oncorhynchus tshawytscha*. FW: fresh water; M: seawater

Test species	Holding conditions	Macerated gill fed (g)		Per os spore dose	No. positive/No. examined					
					Test spp.			Control		
		Test spp.	Control		Fed gill	Per os	Total	Fed gill	Per os	Total
Carassius auratus (avg. 6.0 cm; 3.5 g)	FW	15.0	15.0	8.8×10^5	0/5	0/5	0/10	5/5	5/5	10/10
Cottus asper (avg. 8.8 cm; 8.8 g)	FW	24.0	24.0	2.5×10^6	0/10	0/10	0/20	5/5	5/5	10/10
Cymatogaster aggregata (avg. 8.0 cm; 5.0 g)	M	15.0	15.0	2.5×10^5	0/10	0/10	0/20	5/5	5/5	10/10
Gasterosteus aculeatus (avg. 6.6 cm; 1.7 g)	M	25.0	15.0	1.0×10^5	0/10	0/10	0/20	5/5	5/5	10/10
Oncorhynchus gorbuscha (avg. 17.5 cm; 41.1 g)	FW	24.0	24.0	2.3×10^6	3/10	4/10	7/20	3/5	5/5	8/10
Oncorhynchus keta (avg. 16.8 cm; 36.6 g)	FW	24.0	24.0	4.5×10^6	3/10	8/9	11/19	4/4	3/5	7/9
Poecilia reticulata (avg. 2.7 cm; 0.2 g)	FW	0.5[a]	15.0	1.0×10^5	0/5	0/5	0/10	5/5	5/5	10/10
Salmo salar (avg. 277.8 cm; 193.7 g)	FW	34.0	34.0	1.3×10^6	0/10	0/10	0/20	2/5	5/5	7/10
Salmo trutta (avg. 19.8 cm; 80.3 g)	FW	24.0	24.0	3.5×10^5	10/10	10/10	20/20	5/5	3/5	8/10
Salvelinus alpinus (avg. 13.5 cm; 25.0 g)	FW	13.0	13.0	1.9×10^6	0/10	0/10	0/20	2/5	5/5	7/10
Salvelinus fontinalis (avg. 20.3 cm; 97.2 g)	FW	24.0	24.0	1.9×10^6	2/10	3/10	5/20	5/5	5/5	10/10

[a]Gill lamellae only

Table 2. Prevalence and intensity of *Loma salmonae* in 4 species of salmonid collected from various freshwater watersheds on Vancouver Island, mainland British Columbia, Canada, and the Yukon Territory. NA: prevalence negative by wet mount but positive by PCR

Species	Location	Date	Life history stage of fish	No. fish examined	Prevalence (%)	Intensity (avg. xenomas mm^{-2})
Oncorhynchus nerka (avg. 16.8 cm; 42.2 g)	Great Central Lake[a]	26 Nov 1996	Spawning	66	15.2	0.3
		22 Mar 1997	Smolts	65	8.3 to 41.7[e,f]	NA
(avg. 9.7 cm; 7.5 g)		8 May 1998	Smolts	48	6.3[f]	NA
	Stamp River[b]	24 Jul 1997	Prespawn[d]	33	30.0	0.7
	Sproat River[b]	24 Jul 1997	Prespawn[d]	61	26.2	0.2
	Fulton River[b]	7 Oct 1997	Spawning	63	20.6	0.5
Oncorhynchus kisutch	Big Qualicum River[a]	12 Dec 1996	Spawning	66	3.0	0.4
		4 Dec 1997	Spawning	65	12.3	0.2
Oncorhynchus keta	Big Qualicum River	4 Dec 1997	Spawning	60	1.0	0.2
Oncorhynchus tshawytscha	Yukon River[c]	20 Aug 1997	Spawning	60	0.0	0.0

[a]Vancouver Island
[b]Mainland British Columbia
[c]Yukon Territory
[d]These fish were in fresh water less than 3 d
[e]Prevalence given as a range as 5 fish were combined per sample
[f]Positives detected by PCR

while controls (chinook) were positive (Tables 1 & 2). All salmonid species tested were positive except Atlantic salmon *Salmo salar* and Arctic char *Salvelinus alpinus*. In most cases a higher prevalence was observed using the per os (forced oral feeding) method of infection rather than feeding macerated tissue.

Loma salmonae was detected in wild salmonids sampled from 4 rivers and 1 lake (Table 2). A prevalence of 30.0% occurred in prespawning sockeye that had recently entered fresh water during their return migration to spawn. Intensity of infection was quite high in some fish, and large xenomas were macroscopically visible. None of the sockeye smolts collected on 22 March 1997 and held on ambient seawater for 1 yr were positive by wet mount examination. Furthermore, we were unable to detect *L. salmonae* in newly migrating smolts except by the very sensitive PCR test (Table 2; Figs. 1 to 3).

DISCUSSION

Our ability to infect chum *Oncorhynchus keta* and pink salmon *Oncorhynchus gorbuscha* extends the host range of *Loma salmonae*, demonstrating that the parasite is capable of infecting all 6 species of Pacific salmon in the eastern Pacific. Anonymous (1984) recorded *L. salmonae* in gills and somatic muscle of pink,

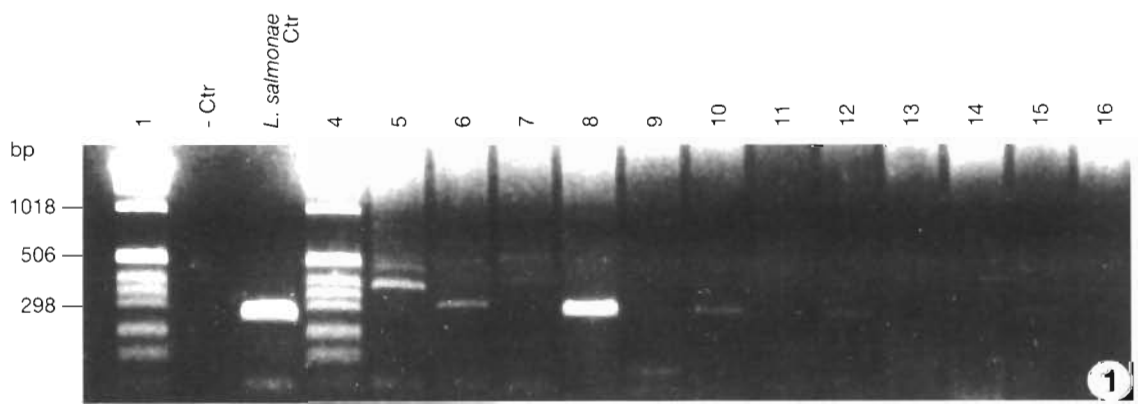

Fig. 1. PCR amplification of DNA from sockeye salmon *Oncorhynchus nerka* gill infected with *Loma salmonae*. Parasite DNA amplified with primers LS-1 and LS-2 (2% agarose gel, ethidium-bromide stained). The negative and positive controls (Ctr) used distilled water and DNA from purified *L. salmonae* spores, respectively. Lanes 5 to 16 are grouped samples of 5 fish per lane. Fish were collected at Great Central Lake, Vancouver Island, British Columbia, Canada, as migrating smolts (1996 year class). Note positives in lanes 6, 8, 10, 12 and 15. Molecular weight markers (bp) are shown in lanes 1 and 4

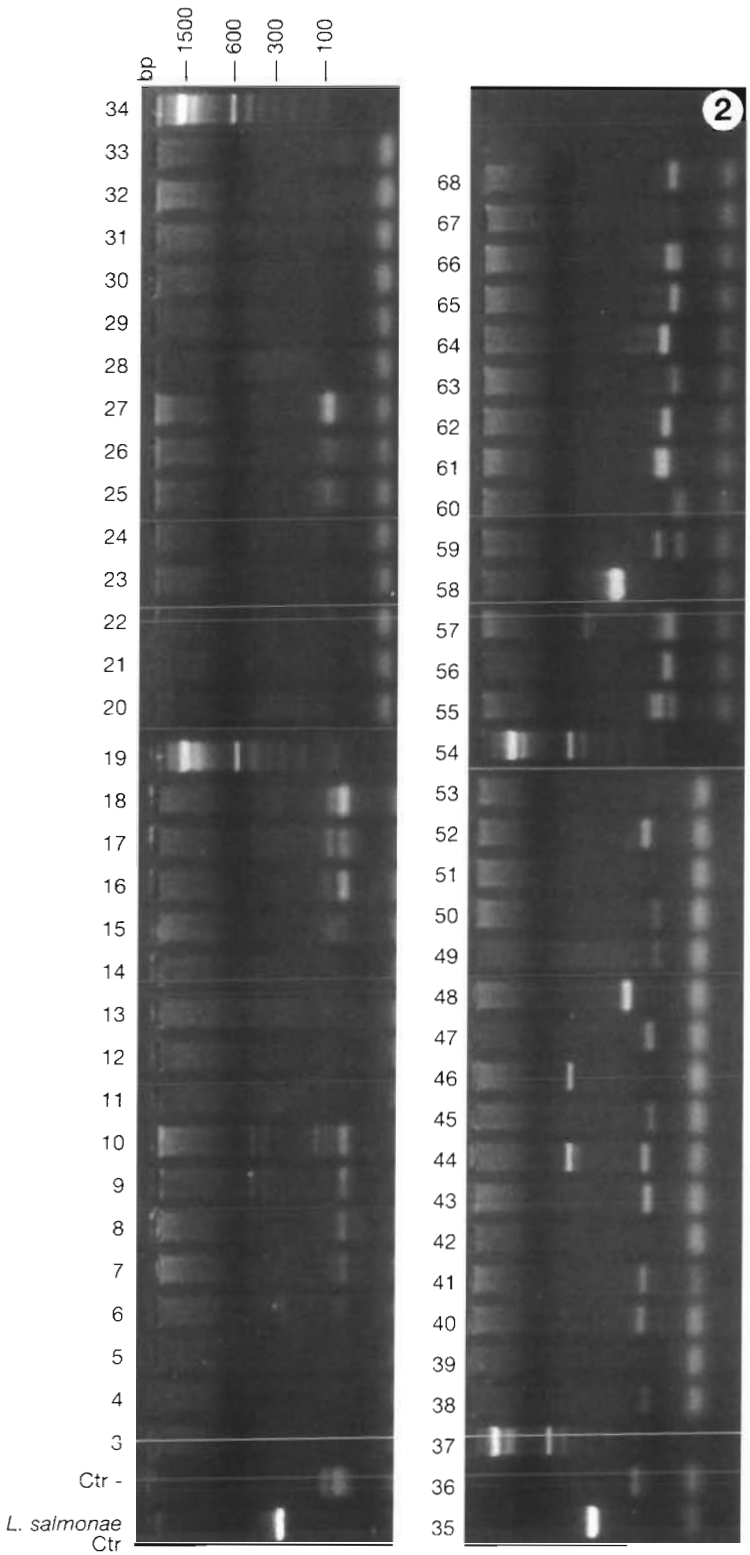

Fig. 2. PCR amplification of DNA from sockeye salmon *Oncorhynchus nerka* gill infected with *Loma salmonae*. Parasite DNA amplified with primers LS-1 and LS-2 (2% agarose gel, ethidium-bromide stained). The negative and positive controls (lanes 1, 35 and 2, 36) used distilled water and DNA from purified *L. salmonae* spores, respectively. Fish were collected at Great Central Lake, Vancouver Island, British Columbia, Canada, as migrating smolts (1997 year class). Note nonspecific bands in lanes 44, 46, 48 and 57 and 1 positive in lane 58. Molecular weight markers (bp) are shown in lanes 19, 34, 37 and 54

chum, and coho salmon from Kemano River, and rainbow trout from Kenny Dam area, British Columbia. They identified spores from stained smears of somatic muscle, suggesting fish were heavily infected. Although this was likely *L. salmonae*, it is possible that this microsporidium was misidentified, as somatic muscle is not a common site of the infection. Our results suggest that reports of *Loma* spp. (not specifically described as *L. salmonae*) from Pacific salmonids (Awakura et al. 1982, Hauck 1984, Magor 1987, Mora 1988, Speare et al. 1989, Gandhi et al. 1995, Kent et al. 1998) are likely *L. salmonae* based on host, parasite morphology, and site of infection.

We were able to infect brook trout with *Loma salmonae*. Bader et al. (1998) reported the ultrastructure and morphology of *L.* cf. *salmonae* as closer to that of *L. salmonae* than *L. fontinalis* Morrison & Sprague, 1983. Bader et al. (1998) could not positively identify the parasite and therefore described it as *L.* cf. *salmonae*. In contrast Speare et al. (1998a) were unable to infect brook trout and suggested the fish used may have been previously exposed to *L. salmonae* or *L. fontinalis*, and, thus, were resistant to re-infection (D. J. Speare, Atlantic Veterinary College, Charlottetown, Prince Edward Island, Canada, pers. comm.) In light of our ability to infect brook trout, *L. fontinalis* may well have been *L. salmonae*. Morrison & Sprague (1983) expressed doubt as to the species validity of *L. fontinalis*. This should be investigated further using molecular systematics. For example, Shaw et al. (1997) found that the internal transcribed spacer region (ITS) of the ribosomal DNA (rDNA) of *L. embiotocia* Shaw, Kent, Docker, Brown, Devlin & Adamson, 1997 differed significantly from that of *L. salmonae*. Furthermore, Brown et al. (1998) showed that the ITS rDNA from isolates of *Loma* from various marine hosts all differed, suggesting that each *Loma* from each marine fish they examined represents a separate species.

We found a low prevalence of *Loma salmonae* in 4 freshwater Pacific salmon species examined, although intensity was quite high in some fish. Kent et al. (1998) reported a maximum prevalence of 41.7% for *Loma* spp. in wild Pacific salmon sampled from seawater. Anonymous (1984) reported a maximum prevalence of *L. salmonae* at 17% and considered the parasite to be typical of post-

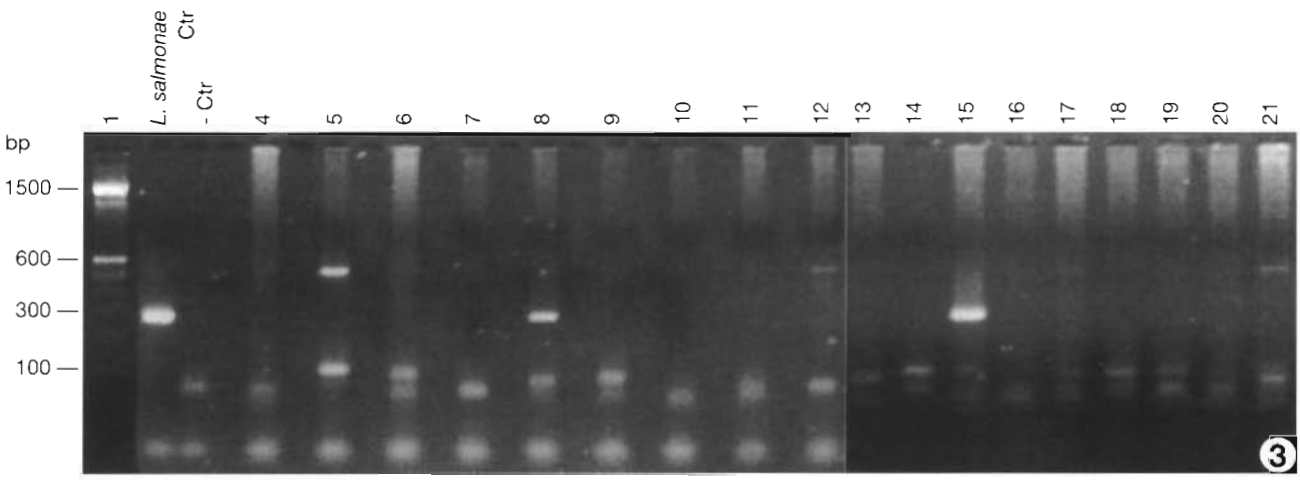

Fig. 3. PCR amplification of DNA from sockeye salmon *Oncorhynchus nerka* gill infected with *Loma salmonae*. Parasite DNA amplified with primers LS-1 and LS-2 (2% agarose gel, ethidium-bromide stained). The negative and positive controls (Ctr) used distilled water and DNA from purified *L. salmonae* spores, respectively. Fish were collected at Great Central Lake, Vancouver Island, British Columbia, Canada, as migrating smolts (≥2 yr old). Note nonspecific bands in lanes 5, 12 and 21 and positives in lanes 8 and 15. Molecular weight marker (bp) is shown in lane 1

spawned fish in the Kemano River. Mortalities in spawning sockeye salmon from the Fulton River, British Columbia, have been associated with high intensity *L. salmonae* infections, and some groups showed as high as 34% prevalence (M. J. Higgins, Fisheries and Oceans, Pacific Biological Station, Nanaimo, British Columbia, pers. comm.).

We detected advanced infections in fish that had recently entered fresh water, and in spawning fish. *Loma salmonae* was also present in smolts migrating out from Great Central Lake. It seems likely that smolts became infected by conspecific freshwater salmonids or by spores deposited with eggs. We were unable to detect *L. salmonae* xenomas by wet mount preparations after holding a sample of sockeye smolts with subclinical infections (i.e. they were positive with the PCR test) for 1 yr on ambient seawater. Docker et al. (1997) found a high prevalence of the infection in the ovaries of sexually mature salmon, and suggested that transmission from adults to progeny may occur. An appealing hypothesis is that fish first become infected as aelvins by spores deposited with eggs. Infected fish may maintain the infection subclinically as they mature during their seawater phase. Shortly before beginning their return migration, and when entering warmer water, fish may begin to develop clinical signs of *L. salmonae*. Conversely, they may recover some months after exposure, and become re-infected either in fresh water or seawater when they undergo sexual maturation. In experimental transmission studies, most fish recover from *L. salmonae* infections a few months after exposure (Speare et al. 1998b, Kent et al. 1999); however, it is unknown how long resistance to re-infection lasts.

In conclusion, we have shown that the host range of *Loma salmonae* now includes all 6 species of Pacific salmon that occur in North America, and brook trout. Of the *Salmo* species, Atlantic salmon are resistant while brown trout are susceptible, indicating that the host range of *L. salmonae* is not absolutely confined to the genus *Oncorhynchus*. Our field investigation has shown that *L. salmonae* is present in various watersheds in British Columbia and throughout the smolt to adult life stages. As members of the genus *Loma* show few distinguishing characteristics at the species level, we suggest that transmission studies and rDNA analysis be employed when describing new species of this specious genus.

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