Journal of Aquatic Animal Health 10:211–219, 1998
American Fisheries Society 1998

Survey of Salmonid Pathogens in Ocean-Caught Fishes in British Columbia, Canada

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Abstract.—A survey of wild fishes captured around marine net-pen salmon farms and from open waters for certain salmonid pathogens was conducted in the coastal waters of British Columbia. Viral hemorrhagic septicemia virus was detected in Pacific herring *Clupea pallasi*, shiner perch *Cymatogaster aggregata*, and threespine sticklebacks *Gasterosteus aculeatus*. Infectious hematopoietic necrosis (IHN) virus was detected in one Pacific herring (collected well away from the farms) and in tube-snouts *Aulorhynchus flavidus* and shiner perch collected from a farm experiencing an IHN outbreak. *Renibacterium salmoninarum* was observed in moribund Pacific hakes *Merluccius productus* collected from within a net-pen and was also detected in several ocean-caught salmon. *Aeromonas salmonicida* subsp. *salmonicida* (typical strain) was isolated from a juvenile chinook salmon *Oncorhynchus tshawytscha*, whereas the atypical strain of this organism was isolated from a lingcod *Ophiodon elongatus*. *Loma salmonae* (Microsporea) was observed in chinook salmon, chum salmon *Oncorhynchus keta*, coho salmon *O. kisutch*, sockeye salmon *O. nerka*, and pink salmon *O. gorbuscha*, all of which were captured well away from net-pens. *Loma* spp. (Microsporea) were observed in the gills of shiner perch, lingcod, Pacific tomcod *Microgadus proximus*, Pacific cod *Gadus macrocephalus*, walleye pollock *Theragra chalcogramma*, and sablefish *Anoplopoma fimbria*; all but the first species represent new hosts for *Loma*. Epitheliocystis, caused by a chlamydia-like organism, was detected in the gills of chinook salmon, chum salmon, coho salmon, pink salmon, lingcod, Pacific cod, Pacific hakes, Pacific tomcod, walleye pollock, sablefish, shiner perch, Dover soles *Microstomus pacificus*, Pacific sanddabs *Citharichthys sordidus*, and various species of rockfish *Sebastes* spp., most of which represent new host records for this infection.

Both net-pen salmon farming and commercial salmon fisheries are important industries in British Columbia, Canada, with landed values of about Can$172 and $276 million, respectively, for 1996. Atlantic salmon *Salmo salar* is the principal species reared in net-pen farms (at 70%), and the remaining production (30%) is mostly chinook salmon *Oncorhynchus tshawytscha*. As with other forms of aquaculture, diseases caused by indigenous pathogens have had a significant economic impact on net-pen farming. The sources (e.g., reservoir hosts) of some of these pathogens are often uncertain, making their avoidance difficult. This is particularly the case with salmon net-pen farming, in which there is unrestricted movement of water through the pens. The Department of Fisheries and Oceans, therefore, initiated a survey of wild marine fishes captured both near salmon net-pens and well away from the farms to provide data on the prevalence and host ranges of certain pathogens of concern in net-pen farming. Fish were examined for the presence of six infectious agents: infectious hematopoietic necrosis (IHN) virus, viral hemorrhagic septicemia (VHS) virus, *Renibacterium salmoninarum*, *Aeromonas salmonicida*, *Loma* spp. (Microsporea), and the epitheliocystis organism.

Methods

Samples were divided into two major categories: fishes collected in open water—well away (>1 km) from marine salmon net-pen farms (henceforth referred to as "farms")—and wild fishes collected from within or around farms (within 0.5 km from the farms). For the former, fishes were collected mostly during cruises of our research vessel, the *W. E. Ricker*, and were collected by bottom trawl. At net-pen farms, fish were collected by hook and line or by hand nets directly from the pens.

Virological assays for IHN and VHS viruses were conducted on combined kidney and spleen tissues in a ratio of approximately 2:1 (weight per weight). Tissues removed from fishes sampled near farm sites were held at 4°C and assayed within 24 h of collection. The tissues from fishes collected during offshore cruises were frozen immediately and stored at −80°C until assayed. All fish were assayed individually by diluting the tissue 1:10 (weight per volume, w:v) with Earle's balanced salt solution prior to homogenizing with a Polytron

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generator (Brinkmann Instruments Co., Rexdale, Ontario, Canada). The homogenized tissues were centrifuged for 10 min at 2,000 × gravity and the supernatant and a 10-fold dilution were inoculated onto preformed monolayers of epithelioma papulosum cyprini (EPC) and chinook salmon embryo (CHSE-214) cell lines. Cell cultures were incubated at 15°C and examined for cytopathic effects for 14 d. Viral isolates were identified by standard neutralization tests or by DNA probes (Batts et al. 1993).

The prevalence of *Renibacterium salmoninarum* was determined by testing frozen kidney tissue with double sandwich polyclonal antibodies in an enzyme-linked immunosorbent assay (ELISA) as described by Pascho et al. (1991). Kidney samples were diluted (w:v) 1:8 for sockeye salmon *Oncorhynchus nerka* and chum salmon *O. keta*, and 1:4 for Pacific herring *Clupea pallasi* and the other fish species. For large groups (samples of 60 fish or greater) of individual species (i.e., adult sockeye salmon or chum salmon), a sample was considered positive if its corrected mean optical density (OD) was 2 SDs greater than the population mean as described by Meyers et al. (1993). For other groups, a sample was considered positive if its uncorrected OD mean value was two SDs greater than the mean of designated negative controls (salmon kidney). Direct fluorescent antibody tests (DFATs) for the organism were conducted on kidney imprints from selected fish as described by Bullock et al. (1980).

Kidneys and gills were examined for the presence of *Aeromonas salmonicida* by culture on tryptic soy agar supplemented with Coomassie brilliant blue (100 mg/L) and 5% fetal bovine serum (Cipriano and Bertolini 1988). Culture plates were held at 15°C for 7 d. Gill tissue was homogenized with a Potter–Elvejhem tissue homogenizer (VWR Canlab, Mississauga, Ontario, Canada) at a concentration of 1:10 (w:v) in sterile saline and then streaked on plates, whereas kidney inocula were obtained directly with sterile swabs. "Atypical" *A. salmonicida* was differentiated from "typical" *A. salmonicida* in that the former produced indole, metabolized sucrose, and produced brown pigment feebly.

The presence of *Loma* spp. or the epitheliocystis organism was determined by histological examination of gills. Gills were preserved in Davidson's fixative and processed by using standard methods. Other selected tissues were examined by histology if fish were moribund or exhibited gross pathological changes.

Results

In all, 2,227 fishes, representing 77 species, were examined from open water collections, and 742 fishes, representing 30 species, were collected around farms. Pathogens or diseases found in these fishes are listed in Table 1.

The VHS virus was detected in clinically normal shiner perch and threespine sticklebacks collected near net-pens. Pacific herring from a wide geographic range of British Columbia were also found to be positive for VHS (confirmed by DNA probes). We detected IHN virus in a Pacific herring collected from Departure Bay, Vancouver Island, in an area not associated with salmon farms. The virus was also detected in tube-snouts and shiner perch collected from an Atlantic salmon farm experiencing an IHN outbreak. The identification of IHN virus in these marine fishes was confirmed by both neutralization tests and with the DNA probe. The virus was not detected in these species collected from the same net-pen site 6 weeks after all Atlantic salmon were removed from the site. Adult sockeye salmon were also found to be infected with IHN virus, as previously reported by Traxler et al. (1997).

Renibacterium salmoninarum was detected by the ELISA method in two of three moribund Pacific hakes collected from a net-pen at a chinook salmon farm, and the presence of this bacterium was confirmed by DFAT in one of these fish. (Five hakes in a second group tested were negative.) However, histological examination of these fish showed no lesions consistent with bacterial kidney disease (BKD). The gills showed heavy infections with embryonated eggs of blood flukes (probably *Aporocotyle margolisi*). Many chinook salmon, chum salmon, coho salmon, sockeye salmon, and Atlantic salmon collected from areas away from farms were also positive for *R. salmoninarum* by ELISA. For the 402 sockeye salmon, 390 fish were examined as two large groups, of which 19 fish were positive. For the 339 chum salmon, 300 fish were examined in one of the groups, 10 of which were positive.

Aeromonas salmonicida subsp. *salmonicida* (typical strain) was cultured from one wild-caught juvenile chinook salmon collected near an Atlantic salmon net-pen farm. Atypical *A. salmonicida* subsp. *salmonicida* was isolated from the kidney of one lingcod collected well away from farms.

Xenomas of *Loma* spp. were detected in several marine fishes, including walleye pollock, Pacific cod, lingcod, Pacific tomcod, sablefish, and shiner

perch. The Pacific cod and walleye pollock (Figure 1) were often heavily infected, exhibiting numerous, macroscopically visible xenomas in the gills and associated chronic inflammation in the primary lamellae. One coho salmon (weight, about 2 kg) collected during one of the *W. E. Ricker* cruises was severely infected with *Loma salmonae*. This fish exhibited renal and splenic swelling and chronic inflammation of the gills associated with numerous xenomas.

Epitheliocystis was observed in several fishes, including salmonids (Table 1). Most infections were light and not associated with significant histological changes. However, one sablefish had a heavy infection in which most of the secondary lamellae were infected (Figure 2).

Discussion

Recently, IHN has caused high mortalities in Atlantic salmon reared in marine net-pens in British Columbia, and field observations suggested that the fish contracted the infections after transfer to seawater (Armstrong et al. 1993; Traxler et al. 1993). The occurrence of IHN virus in marine fishes, particularly in Pacific herring captured well away from farms, supports the hypothesis that nonsalmonid marine fishes may be reservoirs for this virus. Other nonsalmonid fishes have been shown to be susceptible to infection by experimental exposure (Castric and Jeffroy 1991; LaPatra et al. 1995), but our observations of the virus in marine fishes from British Columbia are the first for naturally infected nonsalmonid fishes. The unusual finding of IHN virus in adult sockeye salmon in seawater also suggests the possibility of a seawater reservoir (Traxler et al. 1997). Although these fish were collected in seawater, they were beginning to undergo sexual maturation and soon would have migrated to freshwater to spawn. The IHN virus is commonly found in adult salmon that have returned to freshwater to spawn. However, our observations demonstrate that adult sockeye salmon can express this viral infection while still in seawater. The IHN virus was present in kidney tissues of these fish at levels high enough to indicate that replication was occurring.

Renibacterium salmoninarum is considered to be restricted to salmonid fishes under natural conditions, but nonsalmonid fishes (including Pacific herring, shiner perch, and sablefish) have been experimentally infected (see review by Evelyn 1993). Paclibare et al. (1988) found no *R. salmoninarum* infections in 262 nonsalmonid marine fishes collected around net-pen farms experiencing BKD, and the bacterium was not found in marine fishes collected outside net-pens in our study. However, the occurrence of *R. salmoninarum* in Pacific hakes collected from within a net-pen suggests that this bacterium may occasionally infect certain nonsalmonid fishes when they are confined with *R. salmoninarum*-infected salmon. In this connection, Sakai and Kobayashi (1992) also reported that Japanese sculpin *Cottus japonicus*, flathead *Platycephalus indicus*, and scallops *Platinopectin yessoensis* sampled from a coho salmon farm affected with BKD were found to be positive by serological methods for *R. salmoninarum* antigen. These positive specimens were clinically normal and the bacterium could not be cultured from them. It is, therefore, uncertain whether the bacterium persists in a viable state in these organisms or whether it is rapidly killed, as apparently occurs in other nonsalmonids. For example, experimentally infected common carp *Cyprinus carpio* (see Sakai et al. 1989) and shiner perch (T. P. T. Evelyn, unpublished data) appeared to free themselves of the injected bacteria.

Results from the survey also demonstrated that *Renibacterium salmoninarum* infections are prevalent in Pacific salmon during their ocean phase. A similar finding was reported for salmon collected off the Oregon and Washington coasts in which ocean-caught salmon were examined for *R. salmoninarum* by using a fluorescent antibody test on kidney smears (Banner et al. 1986). The ELISA for *R. salmoninarum* used in our study detects a soluble antigen of the bacterium, and Pascho et al. (1997) demonstrated that the antigen is capable of persisting in uninfected fish for up to 3 months. Therefore, the results from our study may be an overestimate of the prevalence of active infection by the bacterium.

Transmission of another pathogenic bacterium of salmon, *Aeromonas salmonicida*, has occurred in wrasses (family Labridae) maintained together in net-pens with *A. salmonicida*-infected Atlantic salmon (Treasurer and Laidler 1994; Hjeltnes et al. 1995). However, it should be noted that neither *A. salmonicida* nor *R. salmoninarum* were isolated from nousalmonids collected from around net-pen farms. Because *A. salmonicida* occurs in wild (either hatchery-reared or truly wild) salmonids in British Columbia (Hoskins and Hulstein 1977), it is impossible to implicate net-pen farms as the source of the infection in the one wild chinook salmon that was infected with the bacterium. Interestingly, although the infection is not uncommon in juvenile salmonids, it has never been de-

TABLE 1.—Prevalence (number of fish positive for pathogen/number of fish tested) of selected salmonid pathogens in wild-caught fishes from coastal waters of British Columbia near net-pens (N) or offshore (O). Prevalences greater than zero are in bold.

Host (common name and taxon)	VHS[a] virus		IHN[b] virus		*Aeromonas salmonicida*	
	N	O	N	O	N	O
Chinook salmon *Oncorhynchus tshawytscha*		0/96		0/96	1/7	
Chum salmon *O. keta*		0/347		0/347		0/300
Coho salmon *O. kisutch*		0/84		0/84		
Sockeye salmon *O. nerka*		0/436		**7/436**[c]		0/333
Pink salmon *O. gorbuscha*		0/27		0/27		0/10
Atlantic salmon *Salmo salar*		0/53		0/53		0/41
Pacific herring *Clupea pallasi*	**28/128**	**22/161**	0/127	**1/162**[d,e]	0/26	0/49
Northern anchovy *Engraulis mordax*						0/6
American shad *Alosa sapidissima*		0/3		0/3		0/2
Pile perch *Rhacochilus vacca*	0/2		0/2			
Shiner perch *Cymatogaster aggregata*	**10/307**	0/11	**1/307**[d]	0/11	0/110	0/2
Tube-snout *Aulorhynchus flavidus*	0/72		**2/72**[d]		0/21	
Bay pipefish *Syngnathus leptorhynchus*	0/1		0/1		0/1	
Threespine stickleback *Gasterosteus aculeatus*	**6/42**	0/4	0/42	0/4		0/4
Pacific cod *Gadus macrocephalus*		0/34		0/34		0/28
Pacific hake *Merluccius productus*	0/3	0/41	0/3	0/41	0/4	0/33
Pacific tomcod *Microgadus proximus*	0/1	0/10	0/1	0/10		
Walleye pollock *Theragra chalcogramma*		0/23		0/23		0/11
Whitespotted greenling *Hexagrammos stelleri*	0/1	0/7	0/1	0/7	0/1	
Lingcod *Ophiodon elongatus*	0/7	0/24	0/7	0/24	0/2	1/6
Sablefish *Anoplopoma fimbria*	0/3	0/15	0/3	0/15	0/4	0/8
Rockfish species *Sebastes* spp.	0/34		0/34		0/25	
Black rockfish *Sebastes melanops*	0/3		0/3		0/2	
Bocaccio *S. paucispinis*		0/1		0/1		
Canary rockfish *S. pinniger*		0/14		0/14		0/6
Copper rockfish *S. caurinus*	0/14		0/14		0/6	
Darkblotched rockfish *S. crameri*						
Greenstriped rockfish *S. elongatus*						
Pacific ocean perch *S. alutus*		0/6		0/6		0/6
Quillback rockfish *S. maliger*	0/13	0/1	0/13	0/1	0/8	
Redbanded rockfish *S. babcocki*		0/9		0/9		0/9
Redstripe rockfish *S. proriger*		0/37		0/37		0/1
Rosethorn rockfish *S. helvomaculatus*		0/2		0/2		0/2
Rougheye rockfish *S. aleutianus*		0/7		0/7		0/7
Sharpchin rockfish *S. zacentrus*		0/10		0/10		
Silvergray rockfish *S. brevispinis*		0/11		0/11		0/11
Yelloweye rockfish *S. ruberrimus*		0/3		0/3		0/3
Yellowmouth rockfish *S. reedi*		0/16		0/16		0/7
Yellowtail rockfish *S. flavidus*		0/32		0/32	0/3	0/28
Spiny dogfish *Squalus acanthias*	0/2	0/10	0/2	0/10	0/4	0/10
Spotted ratfish *Hydrolagus colliei*	0/1	0/6	0/1	0/6	0/3	0/4
Skate species Rajidae		0/2		0/2		0/2
Sculpin species Cottidae	0/7	0/4	0/7	0/4	0/3	
Threadfin sculpin *Icelinus filamentosus*		0/3		0/3		0/1
Spinyhead sculpin *Dasycottus setiger*		0/1		0/1		
Soft sculpin *Psychrolutes sigalutes*		0/4		0/4		
Roughspine sculpin *Triglops macellus*						
Brown Irish lord *Hemilepidotus spinosus*						
Righteye flounder species Pleuronectidae	0/4		0/4		0/3	0/19
Arrowtooth flounder *Atheresthes stomias*		0/9		0/9		0/2
English sole *Pleuronectes vetulus*						
Dover sole *Microstomus pacificus*						
Flathead sole *Hippoglossoides elassodon*		0/1		0/1		
Pacific halibut *Hippoglossus stenolepis*		0/23		0/23		0/9
Petrale sole *Eopsetta jordani*		0/5		0/5		0/3
Rex sole *Errex zachirus*		0/10		0/10		0/10
Rock sole *Pleuronectes bilineatus*	0/6	0/2	0/6	0/2	0/1	0/2
Slender sole *Eopsetta exilis*		0/6		0/6		
Starry flounder *Platichthys stellatus*	0/2	0/1	0/2	0/1	0/1	
Pacific sanddab *Citharichthys sordidus*	0/3	0/8	0/3	0/8		0/10
Eulachon *Thaleichthys pacificus*		0/4		0/4		
California smoothtongue *Leuroglossus stilbius*		0/1		0/1		

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TABLE 1.—Extended.

Host (common name)	*Renibacterium salmoninarum* N	*Renibacterium salmoninarum* O	*Loma* spp. N	*Loma* spp. O	Epitheliocystis N	Epitheliocystis O
Chinook salmon		**45/77**		**2/82**		**12/82**
Chum salmon		**25/339**		**1/32**		**29/32**
Coho salmon	0/1	**31/74**		**1/48**		**11/48**
Sockeye salmon		**25/402**		**1/15**		0/15
Pink salmon		**7/27**		**5/12**		**1/12**
Atlantic salmon		**17/96**				
Pacific herring	3/19			0/38		**8/38**
Northern anchovy						
American shad				0/3		0/3
Pile perch	0/13		0/2			
Shiner perch	0/1		**12/80**[f]	**1/32**		**13/32**
Tube-snout						
Bay pipefish			0/1			
Threespine stickleback			0/42	0/12		0/12
Pacific cod		0/1		**32/98**		**1/98**
Pacific hake	**2/8**		0/3	0/30		**3/25**
Pacific tomcod			0/1	**3/40**		**1/40**
Walleye pollock				**8/20**		**1/20**
Whitespotted greenling	0/2		0/1			
Longcod			0/7	**19/111**		**16/111**
Sablefish	0/5		0/3	**2/33**		**4/33**
Rockfish species	0/12		0/34			
Black rockfish			0/3			
Bocaccio				0/1		0/1
Canary rockfish				0/12		**8/12**
Copper rockfish			0/14			
Darkblotched rockfish				0/10		**3/10**
Greenstriped rockfish				0/6		**3/6**
Pacific ocean perch				0/7		**4/7**
Quillback rockfish	0/1		0/13			
Redbanded rockfish				0/10		**4/10**
Redstripe rockfish				0/31		**17/31**
Rosethorn rockfish	0/1			0/2		0/2
Rougheye rockfish				0/13		**8/13**
Sharpchin rockfish				0/5		**3/5**
Silvergray rockfish				0/11		**3/11**
Yelloweye rockfish				0/4		**1/4**
Yellowmouth rockfish				0/2		**1/2**
Yellowtail rockfish				0/22		**7/21**
Spiny dogfish	0/3		0/2	0/10		0/10
Spotted ratfish	0/3		0/1	0/6		0/16
Skate species				0/2		0/12
Sculpin species	0/1		0/7			
Threadfin sculpin				0/6		0/6
Spinyhead sculpin				0/2		0/2
Soft sculpin				0/5		0/5
Roughspine sculpin				0/2		0/2
Brown Irish lord				0/1		0/1
Righteye flounder species	0/3		0/4			
Arrowtooth flounder				0/15		**4/15**
English sole				0/6		0/7
Dover sole				0/4		**1/4**
Flathead sole				0/4		**2/4**
Pacific halibut				0/11		0/11
Petrale sole				0/10		0/10
Rex sole				0/1		0/11
Rock sole			0/6	0/3		2/3
Slender sole				0/7		0/7
Starry flounder		1/1	0/2			
Pacific sanddab			0/3			**1/2**
Eulachon				0/5		0/5
California smoothtongue			0/25	0/1		0/1

TABLE 1.—Continued.

Host (common name and taxon)	VHS[a] virus		IHN[b] virus		*Aeromonas salmonicida*	
	N	O	N	O	N	O
Chub mackeral *Scomber japonicus*	0/25		0/25			
Wattled eelpout *Lycodes palearis*		0/3		0/3		
Shortfin eelpout *L. brevipes*		0/1		0/1		0/1
Bigfin eelpout *L. correzianus*		0/3		0/3		
Prickleback species Stichaeidae.		0/1		0/1		
Longsnout prickleback *Lumpenella longirostris*						
Snake prickleback *Lumpenus sagitta*		0/2		0/2		
Pacific sand lance *Ammodytes hexapterus*	0/1	0/10	0/1	0/10		0/6
Saddleback gunnel *Pholis ornata*	0/1		0/1		0/1	
Dwarf wrymouth *Cryptacanthodes aleutensis*						
Giant wrymouth *C. giganteus*		0/1		0/1		
Bigeye poacher *Bathyagonus pentacanthus*						
Blacktip poacher *Xeneretmus latifrons*						
Pygmy poacher *Odontopyxis trispinosa*		0/1		0/1		
Warty poacher *Occella verrucosa*		0/1		0/1		
Northern ronquil *Ronquilus jordani*						
Searcher *Bathymaster signatus*						
Plainfin midshipman *Porichthys notatus*		0/1		0/1		
Northern lampfish *Stenobrachius leucopsarus*		0/8		0/8		

[a] Viral hemorrhagic septicemia.
[b] Infectious hematopoietic necrosis.
[c] Data from Traxler et al. (1997).
[d] Positives confirmed with DNA probe.
[e] Positives confirmed with neutralizing antibody test.
[f] Data from Shaw et al. (1997).

tected in adult salmon captured in seawater. Our results, and those of Nomura et al. (1993), strongly suggest that adult Pacific salmon are free of *A. salmonicida* while in the ocean and that they become infected with the bacterium only after their return to freshwater to spawn.

Loma salmonae has caused disease in pen-reared chinook salmon and coho salmon in the Pacific Northwest (Kent et al. 1989; Speare et al. 1989). At some net-pen farms it appeared that the salmon contracted the infection in seawater, and thus marine fishes in our study were screened for *Loma*

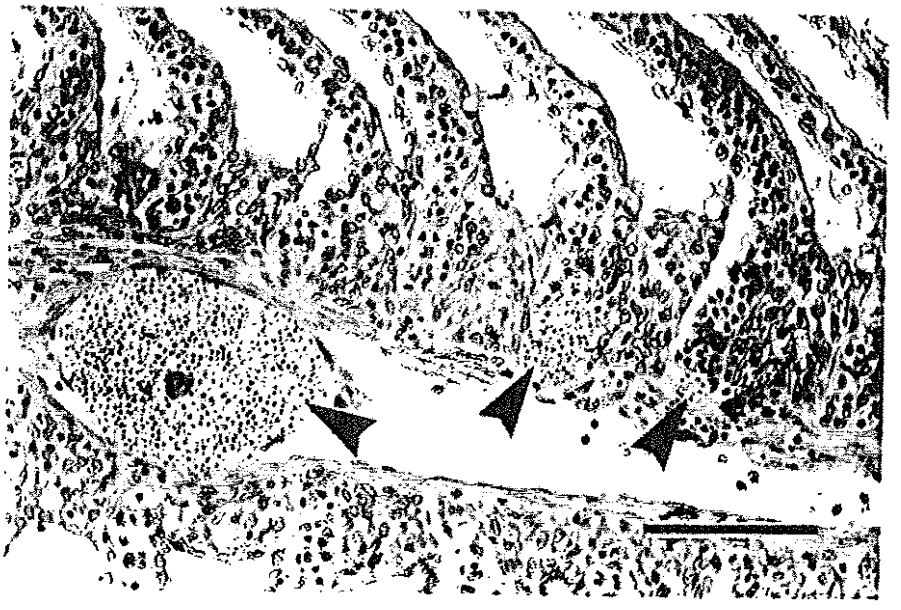

FIGURE 1.—Xenomas of a *Loma* sp. (arrowheads) in the gill of walleye pollock *Theragra chalcogramma*. Bar = 50 µm.

TABLE 1.—Extended, continued.

Aeromonas salmonicida		Host (common name)	*Renibacterium salmoninarum*		*Loma* spp.		Epitheliocystis	
N	O		N	O	N	O	N	O
		Chub mackerel						
		Wattled eelpout						
	0/1	Shortfin eelpout				0/6		0/6
		Bigfin eelpout				0/11		0/11
		Prickleback species						
		Longsnout prickleback				0/2		0/2
		Snake prickleback						
	0/6	Pacific sand lance			0/1			
0/1		Saddleback gunnel			0/1			
		Dwarf wrymouth						0/2
		Giant wrymouth						
		Bigeye poacher						0/1
		Blacktip poacher						0/3
		Pygmy poacher				0/1		0/1
		Warty poacher						
		Northern ronquil						0/4
		Searcher						
		Plainfin midshipman				0/1	1/1	
		Northern lampfish					0/10	

d disease in pen-reared salmon in the Pacific 9; Speare et al. 1989). peared that the salmon seawater, and thus ma- re screened for *Loma*

spp. in an attempt to identify potential reservoirs for the infection. *Loma* spp. have been reported from many marine fishes, including members of the family Gadidae (Canning and Lom 1986). Our observations in the present study are the first reports of *Loma* spp. in Pacific cod, lingcod, walleye pollock, sablefish, or Pacific tomcod. The *Loma* species from shiner perch was recently described by Shaw et al. (1997) and named *L. embiotocia*. Docker et al. (1997) developed a sensitive polymerase chain reaction (PCR) test for *L. salmonae* that used primers to amplify the ribosomal DNA. By using these primers and sequence comparisons, Shaw et al. (1997) established that *L. embiotocia* of shiner perch (which is morphologically very similar to *L. salmonae*) is a different species. Furthermore, attempts at transmitting *L. salmonae* to shiner perch have been unsuccessful (Kent et al. 1995). As with *L. embiotocia*, the *Loma* spores found in other marine fishes were morphologically indistinguishable from those of *L. salmonae*. Therefore, the ribosomal DNA of these *Loma* isolates, as well as that from *L. morhua* from Atlantic cod *Gadus morhua* and *L. branchialis* from had-

a chalcogramma. Bar =

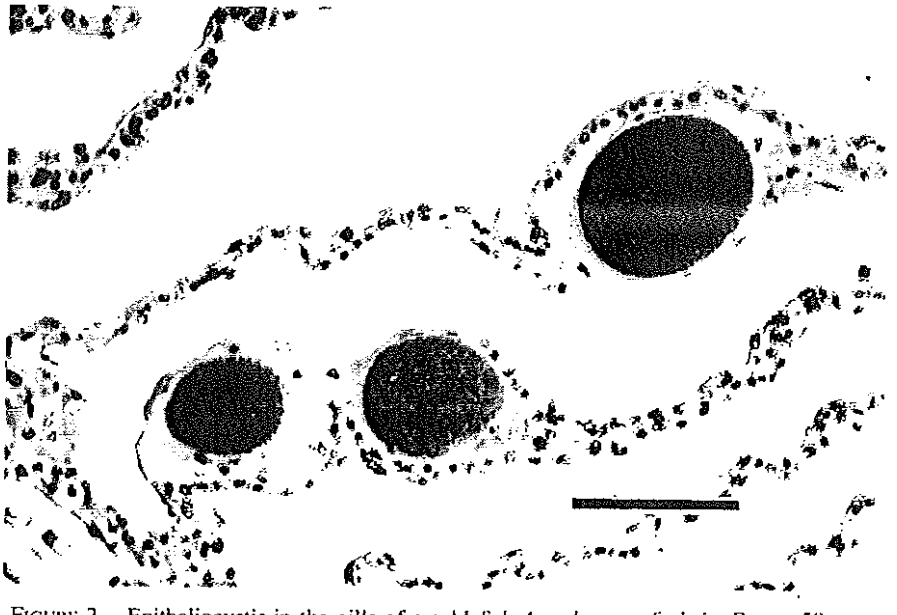

FIGURE 2.—Epitheliocystis in the gills of a sablefish *Anoplopoma fimbria*. Bar = 50 μm.

dock *Melanogrammus aeglefinus* will be analyzed (A. Brown and M. Adamson, Department of Zoology, University of British Columbia, personal communication) in an attempt to clarify their relationships to each other and to *L. salmonae*.

Epitheliocystis, caused by a chlamydia-like agent, is a common disease of salmonid fishes as well as many other fishes (Wolf 1988; Lewis et al. 1992; Fryer and Lannan 1994). Thorough taxonomy studies have not been conducted on the agent of this lesion, and it is possible that the infection is caused by an assemblage of related microorganisms rather than by one species (Turnbull 1993). The present report adds several new hosts for this agent. Although epitheliocystis was common in our survey, the associated pathologic changes were usually minor. If epitheliocystis is caused by one agent, then these marine fishes may serve as reservoir hosts for the infection in pen-reared salmon. The infection has been observed in pen-reared salmon in Norway (Bruno and Poppe 1996), but it has not been reported from pen-reared salmonids in the Pacific Northwest.

The data reported here represent the results to date of a continuing survey of wild marine fishes in British Columbia. This study further substantiates recent observations by others (Meyers et al. 1992, 1994; Meier et al. 1994) that some pathogens thought to be restricted to salmonids have a broader host range, and it expands the list of pathogens important to salmon farming for which wild marine fishes may act as reservoirs. It should be noted that this study represents a survey for infection, not presence of disease, and that almost all of the fish in the study were clinically normal. Although it is well documented that wild fishes may act as reservoirs for certain pathogens afflicting net-pen farms, it should be clearly noted that the data presented here do not indicate transfer of pathogens from net-pens to wild fish populations (or vice versa). In addition to continuing our survey, we are conducting laboratory transmission studies with these pathogens from marine fishes to determine their ability to infect and cause disease in salmon.

Acknowledgments

We thank the fish farmers of British Columbia and the crew of the research vessel *W. E. Ricker* for their assistance with this study. This survey was funded in part by a grant through the Department of Fisheries Reallocation/High Priority program.

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