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SURVEY OF PARASITES IN THREATENED STOCKS OF COHO SALMON (*ONCORHYNCHUS KISUTCH*) IN OREGON BY EXAMINATION OF WET TISSUES AND HISTOLOGY

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ABSTRACT: We are conducting studies on the impacts of parasites on Oregon coastal coho salmon (*Oncorhynchus kistuch*). An essential first step is documenting the geographic distribution of infections, which may be accomplished by using different methods for parasite detection. Thus, the objectives of the current study were to (1) identify parasite species infecting these stocks of coho salmon and document their prevalence, density, and geographic distribution; (2) assess the pathology of these infections; and (3) for the first time, determine the sensitivity and specificity of histology for detecting parasites compared with examining wet preparations for muscle and gill infections. We examined 576 fry, parr, and smolt coho salmon in total by histology. The muscle and gills of 219 of these fish also were examined by wet preparation. Fish were collected from 10 different locations in 2006–2007. We identified 21 different species of parasites in these fish. Some parasites, such as *Nanophyetus salmincola* and *Myxobolus insidiosus*, were common across all fish life stages from most basins. Other parasites, such as *Apophallus* sp., were more common in underyearling fish than smolts and had a more restricted geographic distribution. Additional parasites commonly observed were as follows: *Sanguinicola* sp., *Trichodina truttae*, *Epistylis* sp., *Capriniana piscium*, and unidentified metacercariae in gills; *Myxobolus* sp. in brain; *Myxidium salvelini* and *Chloromyxum majori* in kidney; *Pseudocapillaria salvelini* and adult digenean spp. in the intestine. Only a few parasites, such as the unidentified gill metacercariae, elicted overt pathologic changes. Histology had generally poor sensitivity for detecting parasites; however, it had relatively good specificity. We recommend using both methods for studies or monitoring programs requiring a comprehensive assessment of parasite identification, enumeration, and parasite-related pathology.

Over the last half century, populations of wild salmonids have been declining at alarming rates (Lackey et al., 2006). Currently, Oregon coastal coho salmon (*Oncorhynchus kistuch*), an evolutionarily significant unit set by the National Oceanic and Atmospheric Administration Fisheries, are listed as threatened under the U.S. Endangered Species Act. Therefore, we are conducting studies to elucidate the impacts of parasites on these threatened populations of coho salmon. As a first step, we sought to identify the parasite species infecting these stocks of coho salmon and to document their prevalence, density, and geographic distributions. Furthermore, we conducted a pathology assessment, which provides data on impacts of parasites at the tissue and organism level (Feist and Longshaw, 2008).

The methods used for surveying parasite infections in wild populations include gross examination, microscopic evaluation of tissues in wet mounts, histology, serology, culture, and polymerase chain reaction (PCR)-based techniques. However, currently there are no specific PCR or serologic tests for most parasites of wild fishes. Thus, most fish surveys rely on identification of parasites from wet tissues, because whole fresh parasites usually provide the best morphologic information for identification (Hoffman, 1999; Ferguson, 2006) and permit enumeration of parasites (Hoffman, 1999; Jacobson et al., 2008, Ferguson et al., 2010). Examples of such surveys with salmonid fishes include Kent et al. (1998) and Arkoosh et al. (2004). Histology, however, remains useful as a general tool for conducting initial fish health surveys because it provides the best representation of pathologic changes and allows for detection of a wide variety of pathogens, including viral and bacterial agents (Kent et al., 1998; Feist and Longshaw, 2008). Indeed, most well recognized or common parasites can be readily identified by histology (Gardiner et al., 1998; Gardiner and Poynton, 1999; Bruno et al., 2006). However, aside from a few of our studies (Eaton et al., 1991; Moran and Kent, 1999; Kent et al., 2005), we are not aware of surveys that have evaluated fish by both histology and examination of wet material. Furthermore, we could not find any studies that have empirically compared these 2 methods for detecting parasites.

The Oregon Department of Fish and Wildlife (ODFW) manages life-cycle monitoring (LCM) sites for estimating fish survival as part of the state's plan for assessing the status of threatened coastal stocks (Solazzi et al., 2000). We have shown previously that parr coho salmon from 1 of these LCM sites, West Fork Smith River, have remarkably high digenean and myxozoan infections by histology (Rodnick et al., 2008). Here, we examined coho salmon from several LCM sites in western Oregon by using histology, wet preparations, or both, from fry, parr, and smolt stages. The objectives of our study were to (1) identify parasite burdens and distributions in juvenile coho salmon; (2) assess the pathology of these infections; and (3) for the first time, determine the sensitivity and specificity of histology for detecting parasites in axial skeletal muscle and gills compared with examining wet preparations. Here, we report our results from this survey, summarize histopathologic findings, and compare the different methods used for detecting parasite infections.

MATERIALS AND METHODS

Sampling fish

Coho salmon, because of their threatened status, were opportunistically sampled by ODFW from their LCM sites (for map, see Suring et al., 2009). Fry and parr were caught by electrofishing pools during ODFW snorkeling surveys, and smolts were captured in screw traps during out-migration. In May and June 2006, fry were sampled from the following 8 ODFW LCM sites: lower main stem of West Fork (WF) Smith River (R) (43°48′54.11″N, 123°46′12.49″W), Mill Creek (Ck) Siletz R (44°44′44.89″N, 123°47′35.72″W), East Fork (EF) Trask R (45°24′55.93″N, 123°36′7.30″W), Cascade Ck of Alsea R (44°19′11.15″N, 123°50′50.73″W), Upper North Fork (UNF) (45°48′54.34″N, 123°41′34.50″W) and Lower North Fork (LNF) (45°48′43.13″N, 123°44′45.55″W) of Nehalem R, and the Upper Main Stem (UMS) (44°14′43.26″N, 123°38′28.38″W) and EF (44°14′57.05″N, 123°37′58.55″W) in the Lobster Ck sub-basin of Alsea R. In September of

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2006, parr (same year class) were sampled from the same locations, excluding the Lobster Ck sites and including an additional site of Winchester Ck of the South Slough of Coos Bay (43°16′55.84″N, 124°19′11.23″W). In April and May 2007, smolts (same year class) were sampled from all of the locations that parr and fry were sampled from previously, plus the additional site Mill Ck of Yaquina R (44°35′5.95″N, 123°54′11.75″W), was included. Finally, in September of 2007, parr (different year class) were sampled from the lower main stem of WF Smith R (43°51′38.96″N, 123°44′59.59″W). All fish were taken by ODFW under their permits.

Histology

Samples for histologic analysis were fixed in Dietrich's solution (30 ml of 95% ethanol, 10 ml of formaldehyde, 2 ml of glacial acetic acid, and 58 ml of distilled water). Samples were processed using standard histology techniques and stained with hematoxylin and eosin (H&E), or Giemsa. Sagittal sections were made from intact fry. Parr and smolts required necropsy and removal of the following organs: liver, spleen, mesonephros kidney, heart, intestine, pyloric cecae, brain, gonad, gill, and axial skeletal muscle (henceforth referred to simply as muscle). These organs were trimmed into 1–2-cm^2 pieces, embedded, and sectioned. One section from each fish was evaluated for parasites. Density of infection (parasites/histologic section of infected fish) was determined by counting all parasites in a section by using standard light microscopy. Myxozoans were enumerated by counting pseudocysts or spore aggregates rather than individual spores, because these all arose from a single progenitor. Only prevalence was recorded for capillarid nematodes in the gut because cross sections of single worms were possible; thus, we could not determine whether counts represented more than 1 worm.

Infection comparisons

Statistical comparisons were made among all 3 fish life stages across all samples sites. Comparison between sample sites was limited to descriptive assessments due to lack of replication of sampling for most watersheds. Only data from histologic evaluations were used because wet material for fry and parr were not available for comparisons. Mean densities of parasites in fry, parr, and smolts were compared with a non-parametric bootstrap *t*-test with 100,000 replications, because data were not normally distributed. Fisher's exact tests were used to test differences in prevalence of parasites among fry, parr, and smolts. All statistical procedures were performed with quantitative parasitology (Rózsa et al., 2000), and significance was set at $P < 0.05$. P values are 2-tailed.

Wet preparations

We were able to obtain fresh or fresh frozen material from smolts collected in April 2007 (brood year 2006) from all sites and parr in October 2007 (brood year 2007) from WF Smith R. Gills were evaluated as warranted by standard practices, and muscle also was included because we were particularly interested in the density of muscle parasites given the severity of infections seen in our previous study (Rodnick et al., 2008). All wet preparations were evaluated at ×100 magnification by using a compound light microscope.

For gill tissue, 2 to 3 gill arches (including gill rakers) were collected from each fish at field sites or the laboratory and examined in wet mounts for parasites. Photomicrographs of representative parasites were obtained from gill preparations from fish that were examined in the laboratory. A few metacercariae were excysted in the laboratory as described by Irwin (1983) to better study morphology. Fish from sampling sites that were processed in the laboratory included smolts from Cascade Ck and Mill Ck Yaquina and parr (brood year 2007) from WF Smith R.

For muscle tissue, samples were prepared and evaluated in accordance with previously published methods by Ferguson et al. (2010). In short, parasites were enumerated from 1 freshly thawed and squashed fillet per fish and recorded in terms of parasites/fillet. Also, a gross examination of all fresh or frozen fresh fish was performed to enumerate neascus (black spot trematode) infecting skin and recorded in terms of parasites/fish. Prevalence (number of infected animals/total animals), intensity (number of parasites/infected animals), and density (number of parasites/quantity of infected tissue) of infections are reported in accordance with the definitions provided by Bush et al. (1997). Gill wet mounts and muscle squashes are both referred to as wet preparations henceforth.

Parasite identifications

Parasites were identified as specifically as possible by recording morphologic characters, measurements, and tissue distribution and referring to reviews and keys including Love and Moser (1983), Lom and Dyková (1992), McDonald and Margolis (1995), Gibson (1996), Hoffman (1999), and Moles (2007).

Diagnostic method comparison

We calculated the sensitivity and specificity of histology by using results from wet tissue preparation evaluations as our selected reference test, because this is the most common method used by parasitologists to enumerate and identify parasites in tissues (Hoffman, 1999; Ferguson, 2006). Sensitivity was calculated by dividing the number of fish found positive by both wet preparation and histology by the total number of positive fish by wet preparation. Specificity was calculated by dividing the number of fish found negative by both wet preparation and histology by the total number of wet mount-negative fish. The 95% confidence intervals for the sensitivity and specificity of histology were determined for each parasite by using Clinical Calculator 1 (Lowry, 2010). Only smolts and parr from WF Smith R (brood year 2007) could be included in our comparison analysis of the diagnostic test methods because these were the only samples that were assessed by both techniques.

We also calculated the sensitivity of histology for each muscle parasite when only densely infected fish (top 10% of infections) and negative fish were included in the study to assess the accuracy of this diagnostic technique for detecting densely parasitized fish. This was not performed on gill infections, as there were too few fish with heavy infections.

RESULTS

Total fish evaluated

We evaluated 576 coho salmon (254 fry, 118 parr, and 204 smolts) from 10 coastal rivers in Oregon from 2006 to 2007. All groups were evaluated by histology, but wet preparations of gill and muscle also were evaluated for all smolt groups and 1 parr group. Most fish were infected with several protistan, myxozoan, and helminth parasites (Tables I–III) as we described below.

Muscle parasites

Using both methods, *N. salmincola* was observed in muscle of fish from at least 1 life stage at 8 sites. Based on histology, prevalence and mean density of *N. salmincola* in muscle were similar in fry, parr, and smolts (Table I). High prevalence and infection densities occurred in fry and parr from most locations (Table I). With wet preparations, prevalence of *N. salmincola* in muscle was 100% from smolts from Mill Ck. Siletz, WF Smith R, and LNF Nehalem, with a high density occurring in smolts from WF Smith R. We did not detect *N. salmincola* in any stage of fish from the EF Trask by either method, and it was only detected at a low prevalence and density with wet preparations for smolts from EF Lobster Ck. In histologic sections, *N. salmincola* was observed throughout muscle, multiple visceral organs, and even gills of smolts (Fig. 1B). The kidney was particularly densely infected. Metacercariae were usually surrounded by mild, focal, chronic inflammation.

Using both methods, *Apophallus* sp. was observed in muscle of fish from at least 1 life stage at 7 sites. In contrast to *N. salmincola*, prevalence of *Apophallus* sp. significantly declined throughout all three fish life stages ($P < 0.05$; Fisher's exact test), with smolts having almost 20 times less prevalence than fry (Table I). Hence, using histology, this parasite was only detected in smolts at LNF Nehalem and Cascade Ck (Table I). Almost all

TABLE I. Prevalence and mean density (in parentheses) of parasites in axial skeletal muscle of coho salmon by 2 different methods. Only histology was available for fry and parr (brood year 2006), whereas histology and wet tissues were evaluated for smolts (brood year 2006) and parr (brood year 2007). Histo = histology, Wet = wet preparations, EF = East Fork, R = River, Ck = Creek, WF = West Fork, UNF = Upper North Fork, LNF = Lower North Fork, UMS = Upper Main Stem. Mean density data from histology refer to mean number of parasites/histologic section (1 section of muscle/fish), and mean density data from wet preparations refer to mean number of parasites/fillet. For neascus, Gross = gross examination, and mean intensity (in parentheses) refers to mean number of parasites/infected fish.*

	Nanophyetus salmincola		*Apophallus* sp.		*Myxobolus insidiosus*		*Myxobolus fryeri*	Neascus (in skin)
Location and fish stage	Histo	Wet	Histo	Wet	Histo	Wet	Histo	Gross
EF Trask R								
Fry (n = 35)	0	NA†	0	NA	0	NA	0	NA
Parr (n = 20)	0	NA	0	NA	0	NA	0	NA
Smolts (n = 20)	0	0	0	0	0	90 (10)	0	0
Mill Ck (Siletz)								
Fry (n = 30)	93 (3)	NA	80 (1)	NA	0	NA	0	NA
Parr (n = 20)	85 (6)	NA	20 (3)	NA	5 (1)	NA	30 (10)	NA
Smolts (n = 21)	5 (1)	100 (17)	0	86 (4)	19 (7)	100 (199)	43 (5)	24 (7)
WF Smith R								
Fry (n = 29)	59 (3)	NA	79 (3)	NA	0	NA	0	NA
Parr (n = 10)	60 (3)	NA	40 (2)	NA	60 (26)	NA	10 (6)	NA
Smolts (n = 24)	17 (1)	100 (22)	0	95 (24)	29 (4)	75 (9)	4 (8)	4 (15)
Parr, 2007 (n = 15)	91 (4)	100 (28)	100 (6)	100 (522)	91 (30)	87 (147)	45 (15)	20 (3)
UNF Nehalem R								
Fry (n = 30)	13 (1)	NA	47 (2)	NA	0	NA	0	NA
Parr (n = 20)	55 (2)	NA	35 (2)	NA	0	NA	5 (3)	NA
Smolts (n = 20)	60 (5)	80 (7)	0	45 (2)	25 (5)	100 (21)	40 (7)	15 (4)
LNF Nehalem R								
Fry (n = 60)	58 (2)	NA	37 (6)	NA	0	NA	0	NA
Parr (n = 18)	28 (2)	NA	39 (2)	NA	0	NA	0	NA
Smolts (n = 20)	30 (2)	100 (11)	10 (2)	60 (20)	10 (2)	80(6)	20 (7)	40 (7)
Cascade Ck								
Fry (n = 10)	50 (1)	NA	10 (3)	NA	0	NA	0	NA
Parr (n = 10)	40 (2)	NA	80 (4)	NA	0	NA	0	NA
Smolts (n = 20)	20 (3)	67 (4)	5 (1)	10 (24)	0	100 (70)	0	10 (8)
EF Lobster Ck								
Fry (n = 30)	0	NA	0	NA	0	NA	0	NA
Smolts (n = 20)	0	5 (2)	0	0	0	80 (12)	0	0
UMS Lobster Ck								
Fry (n = 30)	83 (3)	NA	10 (2)	NA	0	NA	0	NA
Smolts (n = 19)	42 (1)	79 (10)	0	0	5 (1)	79 (14)	0	0
Winchester Ck								
Parr (n = 20)	10 (2)	NA	0	NA	50 (10)	NA	10 (5)	NA
Smolts (n = 20)	0	60 (2)	0	0	10 (5)	90 (23)	0	15 (1)
Mill Ck (Yaquina)								
Smolts (n = 20)	15 (1)	80 (2)	0	20 (2)	0	100 (56)	5 (5)	70 (16)
Overall								
Fry (n = 254)	46^{a} (3^{a})	NA	34^{a} (4^{a})	NA	0^{a}	NA	0^{a}	NA
Parr (n = 118)	38^{a} (5^{a})	NA	24^{b} (3^{a})	NA	9^{b} (8^{b})	NA	14^{b} (15^{b})	NA
Smolts (n = 204)	17^{a} (2^{a})	67 (9)	2^{c} (2^{a})	32 (13)	11^{b} (6^{b})	89 (42)	9^{b} (4^{b})	18 (8)

* Parasite prevalence and mean density (in parentheses) in fish life stages with different lowercase superscript letters are significantly different ($P < 0.05$).
† NA = not available.

of the metacercariae of this species were found in myosepta and were not associated with a tissue reaction (Fig. 1A). Using wet preparations, fish from WF Smith R and LNF Nehalem had a high prevalence and density of infection, with the former harboring more than 500 worms/fillet in parr from the lower mainstem section of this river.

The 2 myxobolids infecting muscle were *M. insidiosus* within myocytes, and *M. fryeri* in the peripheral nerves. Using histology,

TABLE II. Prevalence and mean density (in parentheses) of gill parasites of coho salmon by 2 different methods. Only histology was available for fry and parr (brood year 2006), whereas histology and wet tissues were evaluated for smolts (brood year 2006) and parr (brood year 2007). Histo = histology, Wet = wet preparations, EF = East Fork, R = River, Ck = Creek, WF = West Fork, UNF = Upper North Fork, LNF = Lower North Fork, UMS = Upper Main Stem. Mean density data from histology refer to mean number of parasites/histologic section (1 section of 3 gills/fish), and mean density data from wet preparations refer mean number of parasites/sampled gill tissue (3 gills/fish).*

Location and fish stage	*Nanophyetus salmincola*		Unidentified gill metacercariae		*Sanguinicola* sp.		*Trichodina truttae*		*Epistylis* sp.		*Capriniana piscium*		*Ichthyophthirius multifiliis*	
	Histo	Wet	Histo	Wet	Histo	Wet	Histo	Wet	Histo	Wet	Histo	Wet	Histo	Wet
EF Trask R														
Fry (n = 35)	0	NA†	0	NA	0	NA	0	NA	0	NA	0	NA	0	NA
Parr (n = 20)	0	NA	0	NA	0	NA	0	NA	0	NA	0	NA	0	NA
Smolts (n = 20)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mill Ck (Siletz)														
Fry (n = 30)	10 (1)	NA	7 (1)	NA	0	NA	0	NA	0	NA	0	NA	0	NA
Parr (n = 20)	30 (2)	NA	75 (4)	NA	5 (1)	NA	0	NA	0	NA	0	NA	0	NA
Smolts (n = 21)	43 (2)	0	19 (2)	0	0	0	0	5 (1)	0	20 (11)	10 (13)	0	0	15 (2)
WF Smith R														
Fry (n = 29)	10 (1)	NA	10 (1)	NA	0	NA	0	NA	0	NA	0	NA	0	NA
Parr (n = 10)	50 (1)	NA	40 (3)	NA	0	NA	0	NA	0	NA	0	NA	0	NA
Smolts (n = 24)	29 (1)	20 (3)	38 (1)	35 (2)	10 (3)	5 (2)	0	5 (3)	0	0	0	0	0	0
Parr, 2007 (n = 15)	27 (2)	23 (4)	91 (4)	92 (6)	0	0	0	0	0	0	0	0	0	0
UNF Nehalem R														
Fry (n = 30)	0	NA	24 (1)	NA	0	NA	0	NA	0	NA	0	NA	0	NA
Parr (n = 20)	5 (1)	NA	40 (7)	NA	15 (3)	NA	0	NA	0	NA	0	NA	0	NA
Smolts (n = 20)	60 (3)	15 (1)	40 (2)	10 (2)	10 (5)	0	0	5 (1)	0	0	0	0	0	0
LNF Nehalem R														
Fry (n = 60)	0	NA	0	NA	0	NA	0	NA	0	NA	0	NA	0	NA
Parr (n = 18)	0	NA	74 (6)	NA	11 (2)	NA	0	NA	0	NA	0	NA	0	NA
Smolts (n = 20)	65 (3)	20 (3)	25 (3)	5 (5)	10 (7)	0	0	0	0	0	0	0	0	0
Cascade Ck														
Fry (n = 10)	10 (1)	NA	40 (5)	NA	20 (11)	NA	0	NA	0	NA	0	NA	0	NA
Parr (n = 10)	20 (1)	NA	90 (14)	NA	60 (7)	NA	0	NA	0	NA	0	NA	0	NA
Smolts (n = 20)	10 (3)	5 (1)	25 (12)	0	10 (11)	0	0	5 (3)	0	10 (17)	0	0	0	0
EF Lobster Ck														
Fry (n = 30)	0	NA	0	NA	0	NA	0	NA	0	NA	0	NA	0	NA
Smolts (n = 20)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UMS Lobster Ck														
Fry (n = 30)	0	NA	0	NA	0	NA	0	NA	0	NA	0	NA	0	NA
Smolts (n = 19)	5 (1)	5 (1)	0	0	0	0	0	0	0	0	0	0	0	0
Winchester Ck														
Parr (n = 20)	0	NA	0	NA	15 (2)	NA	0	NA	0	NA	0	NA	0	NA
Smolts (n = 20)	0	5 (1)	0	0	45 (8)	0	0	5 (2)	0	0	0	0	0	5 (5)
Mill Ck (Yaquina)														
Smolts (n = 20)	20 (1)	0	55 (4)	0	0	0	0	0	0	0	95 (40)	90 (56)	0	0
Overall														
Fry (n = 254)	3^a (1^a)	NA	6^a (2^a)	NA	1^a (11^a)	NA	0	NA	0	NA	0^a	NA	0	NA
Parr (n = 118)	13^b (1^a)	NA	40^b (7^b)	NA	12^b (3^a)	NA	0	NA	0	NA	0^a	NA	0	NA
Smolts (n = 204)	24^c (2^b)	7 (4)	20^c (4^a)	5 (3)	8^b (7^a)	0.5 (0)	0	3 (2)	0	3 (14)	11^b(27^b)	9 (56)	0	2 (4)

* Parasite prevalence and mean density (in parentheses) in fish life stages with different lowercase superscript letters are significantly different ($P < 0.05$).
† NA = not applicable.

no fry were infected with either species, but parr and smolts from about half the sample sites were infected by both (Table I). The overall prevalence and mean density of infections was comparable in smolts and parr, which was significantly higher than that of uninfected fry ($P < 0.05$; Fisher's exact test and bootstrap *t*-test). Parr from WF Smith R had a high prevalence and density of *M. insidiosus* infections, whereas those from Mill Ck. Siletz had a high prevalence and density of *M. fryeri* infections (Table I). Both

TABLE III. Prevalence and mean density (in parentheses) of viscera or brain parasites of coho salmon by histology for fry, parr, and smolts from brood year 2006 and parr from brood year 2007. EF = East Fork, R = River, Ck = Creek, WF= West Fork, UNF = Upper North Fork, LNF = Lower North Fork, UMS = Upper Main Stem. Mean density data refer to mean number of parasites/histologic section (1 section/fish).

Location and fish stage	*Nanophyetus salmincola*	*Myxobolus* sp. in brain	*Myxidium salvelini*	*Chloromyxum majori*	*Pseudocapillaria salvelini**	Adult digenean spp.
EF Trask R						
Fry (n = 35)	0	0	6 (9)	0	0	17 (1)
Parr (n = 20)	0	25 (2)	0	0	0	5 (1)
Smolts (n = 20)	0	0	0	0	0	5 (1)
Mill Ck (Siletz)						
Fry (n = 30)	97 (5)	0	67 (4)	0	0	80 (3)
Parr (n = 20)	100 (10)	0	0	5 (3)	25	50 (2)
Smolts (n = 21)	86 (13)	0	0	0	50	24 (1)
WF Smith R						
Fry (n = 29)	76 (4)	0	45 (4)	0	0	76 (4)
Parr (n = 10)	80 (6)	40 (10)	0	0	10	50 (1)
Smolts (n = 24)	96 (9)	50 (27)	0	0	33	42 (2)
Parr, 2007 (n = 15)	82 (25)	45 (11)	0	0	85	10 (1)
UNF Nehalem R						
Fry (n = 30)	33 (2)	0	60 (5)	0	0	67 (3)
Parr (n = 20)	95 (1)	85 (11)	0	15 (11)	0	15 (1)
Smolts (n = 20)	90 (9)	30 (12)	0	0	50	65 (5)
LNF Nehalem R						
Fry (n = 60)	77 (4)	2 (1)	52 (3)	23 (8)	0	57 (3)
Parr (n = 18)	28 (5)	50 (6)	0	95 (11)	0	15 (1)
Smolts (n = 20)	95 (42)	60 (6)	0	0	50	60 (3)
Cascade Ck						
Fry (n = 10)	50 (3)	56 (13)	0	30 (3)	0	0
Parr (n = 10)	100 (9)	80 (3)	0	10 (3)	30	0
Smolts (n = 20)	90 (8)	35 (2)	30 (5)	10 (4)	0	75 (3)
EF Lobster Ck						
Fry (n = 30)	0	0	43 (4)	0	0	23 (5)
Smolts (n = 20)	0	0	0	0	0	15 (1)
UMS Lobster Ck						
Fry (n = 30)	63 (4)	0	0	0	0	37 (4)
Smolts (n = 19)	95 (6)	0	0	0	0	11 (1)
Winchester Ck						
Parr (n = 20)	30 (2)	0	0	20 (4)	25	0
Smolts (n = 20)	65 (2)	0	0	0	30	0
Mill Ck (Yaquina)						
Smolts (n = 20)	80 (4)	0	0	0	15	25 (3)
Overall						
Fry (n = 254)	43^{a} (3^{a})	2^{a} (11^{a})	38^{a} (4^{a})	6^{a} (8^{a})	0^{a}	48^{a} (3^{a})
Parr (n = 118)	61^{b} (6^{b})	38^{b} (6^{a})	0^{b}	21^{b} (9^{a})	11^{b}	18^{b} (1^{b})
Smolts (n = 204)	71^{b} (10^{b})	18^{c} (11^{a})	3^{c} (4^{a})	1^{c} (3^{a})	23^{c}	32^{c} (3^{a})

* For *P. salvelini* infections, only prevalence was recorded because cross sections could represent either single or multiple worms. Parasite prevalence and mean density (in parentheses) in fish life stages with different lowercase superscript letters are significantly different ($P < 0.05$).

parasites were generally common in smolts from these rivers (Table I). These parasites did not elicit any inflammatory changes when plasmodia were intact; however, after plasmodia ruptured, a mild inflammatory response was noted. The size of *M. fryeri* pseudocysts (approximately 30 μm) are approximately 6 times smaller than those of *M. insidiosus* (Ferguson et al., 2008) and hence were too small to detect in wet preparation at ×100 magnification. *Myxobolus insidiosus* was detected in smolts from every site in wet material. Mill Ck Siletz smolts and WF Smith R parr had particularly dense infections, with means of nearly 200 and 150 pseudocysts/fillet, respectively.

The presence of neascus in the skin was only evaluated from smolts and the 1 parr group (Table I) because these were the only samples that were available for fresh gross examination. Fish from most rivers were infected, and smolts from Mill Ck Yaquina had nearly 75% prevalence, with a mean intensity of approximately 15/fish (Table I).

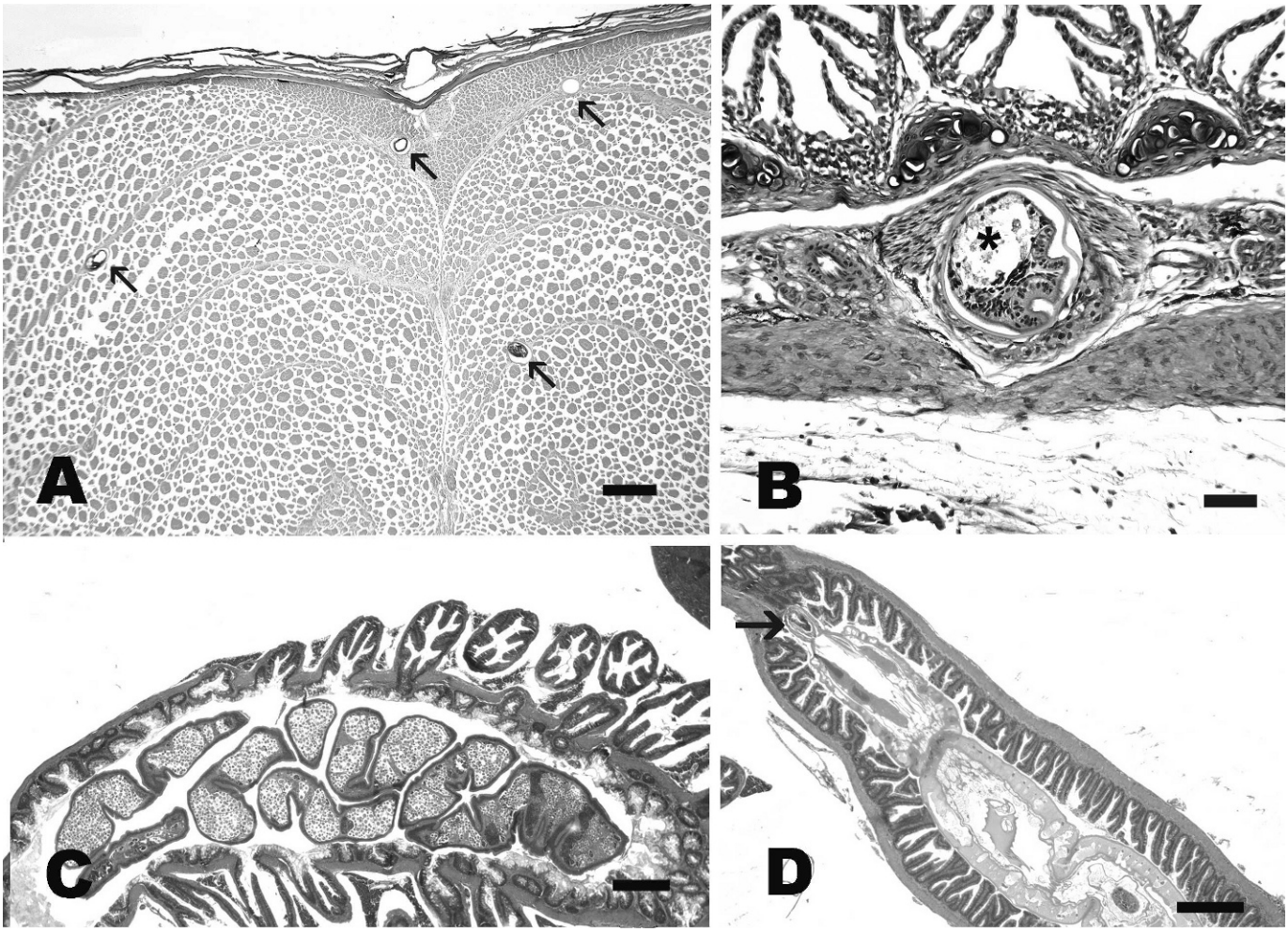

FIGURE 1. Histology showing infection sites of 2 common digenean metacercariae and 2 uncommon adult helminths. (**A**) *Apophallus* sp. in myosepta (arrows), transverse section of axial skelatal muscle. Bar = 200 μm. (**B**) *Nanophyetus salmincola* in gill arch, note prominent excretory bladder (*). Bar = 100 μm. (**C**) Adult tapeworm. Bar = 100 μm. (**D**) Adult acanthocephalan, note proboscis (arrow). Bar = 500 μm.

Gill parasites

Nanophyetus salmincola was observed in the gills of fish from 8 sample sites by histology (Table II). The prevalence of gill infections increased with fish life stages, with smolts having almost 10 times higher prevalence than fry ($P < 0.05$; Fisher's exact test). Smolts also had a significantly higher mean infection density than fry and parr ($P < 0.05$; bootstrap *t*-test). *Nanophyetus salmincola* within the gill filaments was easily recognized by wet preparations by the presence of its opaque posterior excretory bladder (Fig. 2A). However, this structure was not always clearly visible in metacercariae deep in tissues within the base of the gills or gill rakers.

Metacercariae of *Apophallus* sp. and *Echinochasmus milvi* were identified in wet preparations of the gills (Fig. 2C–E). One type exhibited prominent collar spines, consistent with species of Echinostomidae, whereas the other type was a small species (approximately 200 μm long) with a long pharynx, and exhibited features consistent with *Apophallus* sp., including tandem to oblique testes, a pharynx close to the oral sucker, and a long esophagus. However, many of these 2 metacercariae were indistinguishable from each other in histologic sections; hence, we classified them as ''unidentified gill metacercariae.'' Fish from 6 sample sites were infected with these parasites by using this method (Table II), and prevalence and mean density of infections were higher in fry than parr stage by approximately 7 and 4 times, respectfully ($P < 0.05$; Fisher's exact test and bootstrap *t*-test). However, infections were less in parr than smolt stage by approximately half ($P < 0.05$; Fisher's exact test and bootstrap *t*-test). A moderate to high prevalence of infection occurred in both fry and parr from Cascade Ck and a moderate to high prevalence in smolts occurred in Mill Ck Yaquina samples. These unidentified metacercariae elicited the most prominent tissue changes. Located within the base and mid-level of filaments, individual and clustered (2–4 metacercariae) cysts often caused profound intrafilamental fibrosis and concurrent chondroid hyperplasia and metaplasia (Fig. 3A–C). Cysts were surrounded by irregular, pleomorphic, and occasionally binucleated nascent chondrocytes, not directly associated or contiguous with pre-existing cartilage, within a background of dense fibrovascular tissue. The expanded intrafilamental stroma was infiltrated by chronic inflammatory cells, mostly lymphocytes, but also histiocytes and eosinophilic granular cells to a lesser extent.

Embryonated eggs of *Sanguinicola* sp. were detected in histologic sections of fish from 6 sample sites (Table II). Prevalence increased

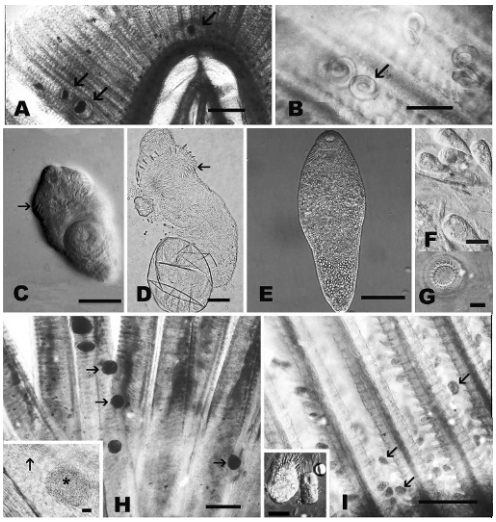

FIGURE 2. Wet preparation examination of parasites. Brightfield or Nomarski's phase contrast. (**A**) Metacercariae of *Nanophyetus salmincola* (arrows) in gill tissue at low magnification. Bar = 500 μm. (**B**) *Philonema* sp. larvae (arrow) in gill tissue. Bar = 250 μm. (**C–D**) *Echinochasmus milvi* metacercariae from gills. Arrows = collar spines. Nomarski's. Bars = 25 μm. (**E**) Excysted metacercariae of *Apophallus* sp. from gill. Nomarski's. Bar = 100 μm. (**F**) *Epistylis* sp. from gills. Normaski's. Bar = 25 μm. (**G**) *Trichodina truttae* from gills. Nomarski's. Bar = 50 μm. (**H**) *Loma salmonae* xenomas (arrows) in gills at low maginifcation. Brightfield. Bar = 250 μm. Inset shows spores at high magnification of xenoma (*) with released spores (arrows). Normaski's. Bar = 10 μm. (**I**) *Capriniana piscium* (arrows) on gill. Brightfield. Bar = 500 μm. Inset shows magnification with characteristic tentacles. Nomraski's. Bar = 10 μm.

from fry to parr stages and then remained the same in smolts ($P < 0.05$; Fisher's exact test). Infections were light and not associated with appreciable tissue damage or inflammation (Fig. 3D). Numerous larvae of *Philonema* sp. were observed in wet preparations of gill from 1 smolt from LNF Nehalem (Fig. 2B). A monogenean suggestive of *Gyrodactylus* sp. also was observed in wet material from a smolt from the WF Smith R.

The following protist parasites were detected in wet preparations of gills from smolts (Table II): *Capriniana piscium* (Fig. 2I, inset), *Trichodina truttae* (Fig. 2G), *Epistylis* sp. (Fig. 2F), and *Ichthyophthirius multifiliis*. *Capriniana piscium* was the only gill protist detected by histology, which occurred on many of the smolts from Mill Ck Yaquina and were not found on fry or parr. These ciliates were observed at the surface of the gill and were not associated with either inflammation or necrosis (Fig. 3E). *Loma salmonae* (Microsporidia) was observed in 2 smolts by wet preparations (Fig. 2H, inset) and 1 smolt by histology (Fig. 3F) from Mill Ck Yaquina, with a mean density of 30 xenomas/gill in examination of wet preparations. Intact xenomas of *L. salmonae* were observed in primary lamellae within major blood vessels and were associated with minimal inflammation. *Trichodina truttae* occurred at about half the sites with a high density in smolts from WF Smith R and Cascade Ck. *Epistylis* sp. and *I. multifiliis* infected smolts from 2 sites, with Mill Ck Siletz harboring high infections (Table II).

Viscera and brain parasites by histology

Additional parasite species were detected when visceral organs and brain were examined by histology. *Nanophyetus salmincola* (common in the muscle) was found in all visceral organs, even the brain and ovary in 1 fish. Including histologic examination of these organs often resulted in doubling the prevalence or mean density of infection compared with examination of gills or muscle only (Tables I–III). Parr and smolts had a higher prevalence and mean density of infections from tissues other than gill and muscle than fry ($P < 0.05$; Fisher's exact test and bootstrap *t*-test).

Aggregates of *Myxobolus* sp. spores were found in the brain of fish (Fig. 3O) from 5 sample sites. Prevalence of these infections was higher in parr than fry stages but then declined in smolts ($P < 0.05$; Fisher's exact test). Fry from Cascade Ck had a high prevalence (Table III). A high prevalence of this parasite also occurred in parr, especially from fish sampled from UNF Nehalem (Table III).

Two kidney myxozoans were observed, i.e., *Myxidium salvelini* and *Chloromyxum majori*, in kidney tubules and glomeruli, respectively. The prevalence of *M. salvelini* was much higher in fry than parr stage ($P < 0.05$; Fisher's exact test) but then increased slightly in smolts (Table III). Conversely with *C. majori*, prevalence was higher in parr than fry stage and then much lower in smolts ($P < 0.05$; Fisher's exact test). *Myxidium salvelini* was confined to the lumen of renal tubules and was not associated with tissue damage. Plasmodia had prominent, retractile granules in the cytoplasm and also contained developing and mature spores (Fig. 3K, L). Myxospores of *M. salvelini* were readily visualized with Giemsa stain (Fig. 3L, inset). Parr at 5 sites and smolts at 1 site (Cascade Ck) were infected with *C. majori* (Table III). This myxozoan was observed in the glomeruli and was identified by the presence of spherical spores with 4 polar capsules (Fig. 3M, N). Affected glomeruli were mostly effaced and expanded by the spores, with significant disruption of normal histoarchitectural features and a narrowing to near-complete obliteration of the uriniferous space associated with attenuated parietal epithelium. In some instances, there was mild periglomerular fibrosis surrounding the affected glomeruli.

Four helminth species were found in the gastrointestinal tract by histology. *Pseudocapillaria salvelini* (nematode) was clearly identified as a capillarid due to the presence of stichosomes and eggs with bipolar plugs (Fig. 3G). Prevalence increased with host age ($P < 0.05$; Fisher's exact test); smolts from Mill Ck Siletz and both forks of Nehalem had high infection prevalences (Table III). For parr, fish from WF Smith R (brood year 2007) had a high prevalence of almost 90%. This nematode occurred within the intestinal epithelium, but was not associated with obvious tissue damage or inflammation.

Unidentified adult digeneans were observed in the lumen of the pyloric cecae of a few fish (Table III) and were not associated with significant histopathologic changes (Fig. 3J). Prevalence of these worms decreased between underyearling stages but increased in smolts ($P < 0.05$; Fisher's exact test), and parr had a significantly lower mean density of infection than fry and smolts ($P < 0.05$; bootstrap *t*-test). Fry from Mill Ck Siletz and WF Smith R had a high prevalence and density of infection, whereas smolts from UNF Nehalem and Cascade Ck had high levels of these indices. An adult cestode was observed in the intestine (Fig. 1C) of 1 fish from EF Lobster Ck and an adult acanthocephalan (Fig. 1D) was observed in the intestine of 4 fish from Winchester Ck. Numerous larval nematodes of *Philonema* sp. were observed in the coelomic cavity and ovary of 1 fish from LNF Nehalem (Fig. 3H, I), which was the same fish we detected larval worms in gill wet preparations. Typically, larvae were

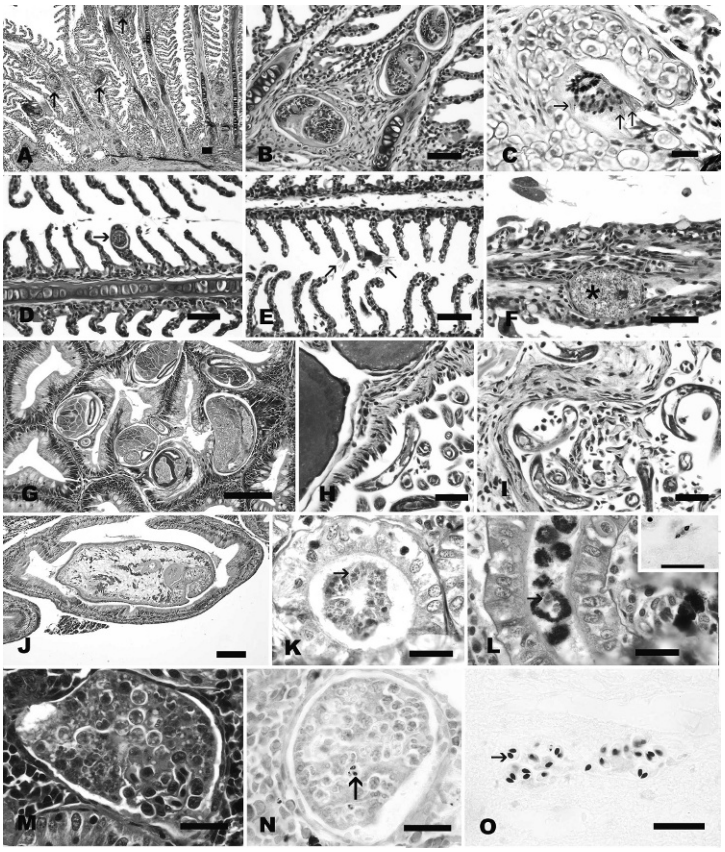

FIGURE 3. Histologic examination of parasites and the tissues they infect. H&E, unless indicated otherwise. (**A–B**) Gill metacercariae (arrow in A) associated with hyperplasia. Bars = 50 μm. (**C**) Metacercaria of *Echinochasmus milvi*, note spines in collar (arrows). Bar = 20 μm. (**D**) Embryonated eggs of *Sanguinicola* sp. (arrow) in gills. Bar = 50 μm. (**E**) *Capriniana piscium* on gills (arrows). Bar = 50 μm. (**F**) *Loma salmonae* in gills (*). Bar = 50 μm. (**G**) *Pseudocapillaria salvelini* in intestine. Bar = 100 μm. (**H–I**) *Philonema* sp. in ovarian tissue. Bars = 100 μm. (**J**) Adult digenean in pyloric ceaca. Bar = 100 μm. (**K–O**) Myxozoans in tissue sections. Bars =25 μm. (**K–L**) *Myxidium salvelini* in kidney tubule. Arrows = spores. Giemsa-stained inset showing dark-staining polar capsule. (**M–N**) *Chloromyxum majori* in kidney glomerulus; N shows polar capsules (arrow) with Giemsa stain. (**O**) *Myxobolus* sp. in brain (arrow), Giemsa stained.

surrounded by many intermixed inflammatory cells, comprised of lymphocytes, histiocytes, and eosinophilic granular cells, with fewer plasma cells being present. Serosal surfaces were lined by hypertrophied reactive serosal cells overlying modest fibroplasia, indicating a low grade and chronic serositis.

Diagnostic method comparison

Using wet preparations as our reference test, the sensitivity of histology was low, i.e., 10–20%, for all the muscle parasites, and some gill parasites were not detected at all (sensitivity test; Table IV). The sensitivity was increased when we excluded muscle tissues with a low mean density of infection (Table IV), but it was still never above 45%. Three gill ciliates, *T. truttae*, *Epistylis* sp., and *I. multifiliis*, were all detected by wet preparation, but they were not found by histology (Table IV). However, histology was good for detecting *C. piscium* infections, with approximately 95% sensitivity (Table IV). Similarly, histology was fairly sensitive (approximately 45%) at detecting gill metacercariae, even though the reference test (wet preparation) consistently missed these infections across many fish populations (compare values in Table II). With the exception of the gill metacercariae, the specificity for histology was high for all other parasites, ranging from 91 to 100% (Table IV). *Sanguinicola* sp. was detected by wet preparation in only 1 group, but it was found in 4 groups in which both histology and wet preparation data were collected (Table II).

DISCUSSION

Study background

Diseases, including those caused by parasites, may be significant contributors to mortality in wild fish populations (Möller and Anders, 1986; Adlard and Lester, 1994; Bakke and Harris, 1998; Jacobson et al., 2008). One of the first steps for assessing impacts of parasites on wild fish populations is to identify the parasite species and to document their prevalence, density, and geographic distribution. We had previously reported heavy helminth and myxozoan infections in coho salmon parr from WF Smith R (Rodnick et al., 2008), consistent with the current survey on fry, parr, and smolt coho salmon from 10 coastal rivers. The parasites found in the present study have been described previously from salmonids in the Nearctic, mostly in parasite surveys or taxonomic descriptions.

Nanophyetus salmincola

One of the most common parasites observed was metacercariae of *N. salmincola*, which were found in all life stages of fish and in

TABLE IV. Sensitivity and specificity of histology, by using evaluations of wet preparations as a standard, for infections in smolts from 10 rivers and parr from 1 river; 95% confidence intervals are in parentheses. Heavy = highest 10% density based on wet preparation results for parasites in axial skeletal muscle. The mean density and (range) of these heavy infections were as follows: *Apophallus* sp. = 309/fillet (16–1,034/fillet), *Nanophyetus salmincola* = 39/fillet (22–61/fillet), and *Myxobolus insidiosus* = 262/fillet (116–570/fillet). Specificity for heavy infections is not presented because they were the same as those that included all data.

	Sensitivity	Specificity
Muscle parasites		
Apophallus sp.	0.15 (0.08–0.25)	0.99 (0.95–1.0)
Apophallus sp., heavy	0.45 (0.25–0.67)	—
N. salmincola	0.26 (0.19–0.34)	0.91 (0.80–0.96)
N. salmincola, heavy	0.32 (0.15–0.55)	—
M. insidiosus	0.13 (0.09–0.19)	1.0 (0.83–1.0)
M. insidiosus, heavy	0.41 (0.21–63)	—
Gill parasites		
Gill metacercariae	0.31 (0.12–0.59)	0.80 (0.74–0.86)
N. salmincola	0.44 (0.21–0.69)	0.77 (0.71–0.83)
Sanguinicola sp.	0 (0–0.37)	0.91 (0.86–0.95)
Trichodina truttae	0 (0–0.54)	1 (0.98–1.0)
Epistylis sp.	0 (0–0.48)	1 (0.98–1.0)
Ichthyophthirius multifiliis	0 (0–0.60)	1 (0.98–1.0)
Capriniana piscium	0.95 (0.72–0.99)	0.98 (0.95–0.99)

all but 1 of the rivers. The intermediate host for this digenean is *Juga silicula*, which is widely spread in coastal streams from northern California through Washington. This snail prefers slower moving water, with coarse substrata (Furnish, 1990), which is consistent with many locations in our study. Prominent infections in the muscle were seen in wet preparations, and this worm was detected in every organ by histology. As reported previously (Wood and Yasutake, 1956; Ferguson et al., 2010), the metacercariae were associated with localized, chronic inflammation.

Apophallus sp.

Metacercariae of *Apophallus* sp. were found in fish from nearly half the sites and was most dense and prevalent in fish from locations with little riparian canopy, because the surrounding lands have been either logged, e.g., WF Smith R, LNF Nehalem, and Cascade Ck, or are used for agriculture, e.g., Mill Ck Siletz (Solazzi et al., 2000). These activities are associated with increased water temperatures in the associated rivers (Beschta and Taylor, 1988), and perhaps this digenean species thrives better under these conditions. For example, summer water temperatures in the lower mainstem of the WF Smith R has often exceeded 20 C (Ebersole et al., 2006), and Rodnick et al. (2008) reported that *Apophallus* sp. metacercariae were more common in coho salmon parr in this stretch of the river compared with cooler, upstream tributaries. The first intermediate hosts for *Apophallus* species infecting different fishes in Oregon are snails of *Fluminicola* spp. (Niemi and Macy, 1974; Kent et al., 2004). Snails (Malek, 1980) and digeneans (Poulin, 2006) increase their reproduction with warmer temperatures. Hawkins et al. (1982) also reported that rivers in Oregon with little riparian canopy, presumably resulting in higher temperatures, had more snails. The abundance of definitive hosts nearby also could be an important factor. For example, the William L. Finley National Wildlife Refuge is approximately 90 km away from the WF Smith R and may allow for many piscivorous birds to frequent the area.

In contrast to *N. salmincola*, we did not observe *Apophallus* sp. in visceral organs. Indeed, the infections in muscle occurred at a specific location, i.e., within the connective tissue of the myospeta. Other reports showed that metacercariae of *Apophallus* species target skin of salmonids (Niemi and Macy, 1974), muscle of percids (Taylor et al., 1994), or bone of cyprinids (Kent et al., 2004). The latter 2 reports involved osseous hyperplasia and metaplasia associated with metacercariae. This was consistent with gill infections in our study, where metacercariae in the gills were often directly associated with chondroid hyperplasia and metaplasia.

Unidentified gill metacercariae

Identification of the metacercariae, other than *N. salmincola*, in the gills was difficult by histology. Occasionally, we observed a distinct spiny collar in histologic sections, a feature consistent with echinostomids. Some excysted worms from wet preparations closely resembled *Echinochasmus milvi*, a common species found in gills of Oregon salmonids associated with similar lamellar damage (Hoffman, 1999). Olson and Pierce (1997) described metacercariae from the gills of Oregon steelhead trout (*Oncorhynchus mykiss*) that caused cartilage proliferation, and concluded that they were probably members of the Heterophyidae. Worms from both families have been noted to induce lamellar hyperplasia (Uzmann and Hayduk, 1964). In our study, chondrocyte hyperplasia and metaplasia associated with metacercariae were similar to that described with gill metacercariae in freshwater tropical fishes (Blazer and Gratzek, 1985). Disease produced by such infections can be serious. For example, gill-infecting *Centrocestus formosanus* (Heterophyidae) causes massive mortalities in cultured warm water fishes (Paperna, 1995).

Sanguinicola sp.

Another digenean found in the gills of juvenile coho salmon was the blood fluke *Sanguinicola* sp. It was not possible to identify this worm to the species level because only eggs and miracidia were observed; however, 2 species infect salmonids in Oregon, *Sanguinicola klamathensis* and *Sanguinicola alseae* (Hoffman, 1999). Embryonated eggs of *Sanguinicola* spp. are found in blood vessels of well-vascularized organs, particularly the gills and kidney. Typical of blood fluke eggs, they elicit local inflammatory changes, and severe infections are associated with morbidity when miracidia escape from gills causing loss of blood and decreasing respiratory function (Schäperclaus, 1991; Paperna, 1995).

Gastrointestinal helminths

A capillarid nematode in the lower intestine of many parr and smolts was found at nearly half the sites. We are confident that worms are *Pseudocapillaria salvelini* (syn. *Capillaria salvelini*, *Ichthyocapillaria salvelini*), because it has been reported from numerous salmonid fishes, including coho salmon from Canada (Bell and Beverly-Burton, 1981). The only other record of a different species in salmonids was *Capillaria catenata* (Fritts, 1959), which typically infects percids and centrarchids. This single

report has been regarded as suspect by Bell and Beverley-Burton (1981), due to its rarity and because the worm was found only in cutthroat trout (*Oncorhynchus clarkii*) and not in percids and centrarchids from the same location. Capillarids are recognized as pathogens in fishes (Dick and Choudhury, 1995), because they penetrate gut tissue and are associated with severe damage and inflammation. For example, *Pseudocapillaria tomentosa* causes severe inflammation and is associated with emaciation and ultimately death in zebrafish (*Danio rerio*; Kent et al., 2002). In contrast, all worms seen in our study were confined to the epithelium and were associated with little, or no, tissue damage.

We did not determine the identities of the adult digeneans observed in the gastrointestinal tract, because there are numerous genera and species infecting salmonids in the Pacific Northwest (Love and Moser, 1983; Hoffman, 1999), and we only observed them by histology. Adult intestinal trematodes are considered to be less pathogenic than metacercariae, because most are confined to the lumen of the gastrointestinal tract (Paperna, 1995). Numerous genera and species of acanthocephalans and cestodes infect salmon and trout in the Pacific Northwest as adult stages (Love and Moser, 1983; Hoffman, 1999; McDonald and Margolis, 1995). We were not able to identify our specimens to the genus level based on the available material.

A severe *Philonema* sp. infection was observed in 1 smolt, with massive numbers of larvae in the viscera and gills. Unfortunately, we were unable to collect adult worms and thus were not able to determine the precise species of this worm. Two genera of philometrids are described from salmonids in the Pacific Northwest, i.e., *Philonema* and *Philometra* (Hoffman, 1999). We identified our worms as *Philonema* sp. based on the tapered posterior end. There are numerous reports of *Philonema oncorhynchi* in salmonids from the Pacific Northwest (Hoffman, 1999; Moravec and Nagasawa, 1999), and thus this may be the species seen here. This parasite is recognized to cause disease in salmonids, including coho salmon (Brocklebank et al., 1996), and the intense inflammatory response seen in the one severely infected fish certainly would have compromised this individual.

Myxozoans

Myxozoans are commonly observed in wild salmonids, and we found 5 species in our survey. *Chloromyxum majori* in the kidney and *Myxobolus* sp. in the brain were common in fish from rivers with high levels of digenean infections. The life cycles of these myxozoans have not been resolved, but based on other myxozoans they probably involve an oligochaete host. Many species of these worms thrive in relatively warm, oligotrophic, or eutrophic environments and this could account for their distribution seen here. In contrast, *Myxobolus insidiosus* infections occurred with a high prevalence and similar density in all rivers, suggesting that the putative oligochaete host for this parasite is widespread. These are general observations, and further studies are required to connect these parasite distributions with ecologic parameters and land use practices.

We observed renal infections by *M. salvelini* in all life stages of coho salmon in 7 of the rivers. Two *Myxidium* species infect the kidneys of salmonids in the Pacific Northwest, i.e., *M. salvelini* and *Myxidium minteri*. The former species has been recorded from urinary bladder, ureter (Shul'man, 1988), and kidney (Shul'man, 1988; Kent et al., 1994; McDonald and Margolis, 1995), whereas the latter has only been found in renal tubules (Yasutake and Wood, 1957; Sanders and Fryer, 1970). We identified the species seen here as *M. salvelini* based on the presence of more elongated spores and pyriform polar capsules. In contrast, the spores of *M. minteri* are oval and polar capsules are subspherical. In addition, one of us (M.K.) has observed *M. salvelini* infections in many salmonids in Canada, and, as seen here, the occurrence of refractile, brown-to-black granules in the cytoplasm of the trophozoites is a consistent finding for this species. Renal tubule degeneration can occur and spores are liberated from tubules and enter into the renal interstitium with heavy infections of *M. minteri* (Yasutake and Wood, 1957), whereas *M. salvelini* is not recognized to be pathogenic (Kent et al., 1994). There was no observable damage to the infected tubules.

Chloromyxum majori infected kidney of fry, parr, or smolts from 5 of the rivers. This species is the only species in this genus that infects glomeruli of salmonids in this geographic region (Yasutake and Wood, 1957; Hoffman, 1999). We found this parasite to predominantly infect the glomerular visceral epithelium and occlude the uriniferous space, with large numbers completely effacing glomeruli. However, it also can cause degeneration of intra-renal hematopoietic tissue in heavy infections that has been associated with salmon losses in hatcheries (Yasutake and Wood, 1957). The glomerular lesions observed here were consistent with previous descriptions, and infected glomeruli were essentially obliterated by the parasite and associated inflammation.

Several neurotropic *Myxobolus* species infect salmonids (for review, see Ferguson et al., 2008). The spores seen here in brains from juvenile coho salmon were probably either *Myxobolus kisutchi* or *Myxobolus neurotropus* because both form oval spores in the brain and spinal cord of salmonids in this region. It is unlikely that these infections were *Myxobolus arcticus*, because this species has a more northerly range and has not been reported in parr or smolts in freshwater south of Washington state (Ferguson et al., 2008). There was minimal tissue reaction to the brain myxobolid, and infections by *M. kisutchi* and *M. neurotropus* have not been associated with disease. However, Moles and Heifetz (1998) found that *M. arcticus*, which causes similar infections, is associated with reduced swimming ability of sockeye salmon (*Oncorhynchus nerka*).

Gill protists

Several protists were observed in, or on, the gills of juvenile coho salmon, including *L. salmonae*, *T. truttae*, *Epistylis* sp., *Capriniana piscium* (syn. *Trichophyra piscium*), and *I. multifiliis*. *Trichodina truttae* was the most prevalent ectoparasitic protist, detected in fish from half of the rivers. This species has been described from salmonids in freshwater in Oregon (Mueller, 1937) and other regions in the Pacific Northwest (Arthur and Margolis, 1984), based on size and morphology. It is pathogenic when it occurs in high numbers, causing epithelial hyperplasia, decreased osmoregulatory ability, and even death (Lom, 1995). We did not observe tissue damage associated with this ciliate.

Both *Epistylis* sp. (Esch et al., 1976) and *C. piscium* (Hofer et al., 2005) have been associated with fish held in water with high organic loads, and certain rivers where these were prevalent, e.g., Siltez R and Yaquinia R, predominantly have agriculture activity

above the sites where fish were collected. The proper identification of epistylids in fishes from North America is lacking (Hoffman, 1999); thus, we refer to the organism seen here as *Epistylis* sp., which is considered more of an ectocommensal than a parasite of both skin and gills. However, high numbers of this organism have been implicated in "red sore disease" with other possible synergistic etiologies (Lom, 1995). Although *Capriniana* infections were very heavy in some fish, with every filament infected with numerous parasites, they were not associated with histologic changes. This is consistent with other reports, where *Capriniana* spp. on salmonid gills and other fishes are considered commensal, rather than parasitic (Lom, 1995; Hofer et al., 2005).

Ichthyophthirius multifiliis is recognized as a serious pathogen (Traxler et al., 1998), but we detected this parasite in just 1 fish. Two fish were infected with the microsporidium, *L. salmonae*, which is a well-known pathogen in net pen aquaculture (Kent and Speare, 2005). Severity of tissue damage is more related to the reaction associated with destruction of xenomas rather than density of infection (Speare et al., 1998). With infections in freshwater, intact xenomas, as seen here, are associated with little damage to host tissues (Magor, 1987).

Evidence for parasite associated mortality

In general, fry and parr were more intensely infected by most parasites than smolts, but a few parasites had a higher prevalence in older fish. There are many examples of temporal declines in parasite burdens of host populations being associated with parasite associated mortality, often involving fishes (for reviews, see Anderson and Gordon, 1982; Lester, 1984; Hudson and Dobson, 1995; Rousset et al., 1996). Recently, Jacobson et al. (2008) reported this to occur with *N. salmincola* infections in coho salmon off the coast of Oregon. Temporal declines in parasite burdens also can be explained by recovery from infections or from non-representative samples of the population from different time points, i.e., smolts are a mixed population of many parr subpopulations. We recently showed that *N. salmincola*, *Apophallus* sp., and *M. insidiosus* persist in coho salmon from parr to smolts (Ferguson et al., 2010); thus, parasite death between parr and smolt stages would not explain the differences seen between these fish life stages in the present study. Although it is possible that the differences in parasite burden was because of the potential nonrandom sampling inherent with studying fish populations, it is unlikely because we observed the same trends in fish sampled across 10 different rivers. High mortality often occurs in coho salmon during the winter preceding smoltification (Ebersole et al., 2006; Hurst, 2007) and pre-overwintering fish from rivers such as WF Smith R have substantially high infections (Rodnick et al., 2008). Therefore, it is most probable that the dramatic declines in parasite burdens in fish from parr to smolt stages, as seen with *Apophallus* sp., are related to parasite associated mortality.

Method comparison

Diagnostic methods for identifying parasites include serology, non-lethal blood and fecal exams, molecular methods, biopsies, and necropsy with examination of tissues by wet mount or histology (Hendrix and Robinson, 2006). With fish, serologic or molecular tests have been developed for some pathogens (Cunningham, 2002; AFS–FHS, 2007), yet there are few that have been developed or verified for helminth parasites. Therefore, the most practical methods for examining fish for a variety of parasites were by histology and wet preparations. Surveys for parasites of wild fishes have been most often based on necropsies and examination of wet tissues (Arthur et al., 1976; Blaylock et al., 1998; Arkoosh et al., 2004; Butorina et al., 2008; Jacobson et al., 2008). However, some studies have relied on, or included, data obtained by histology (Kent et al., 1998; Longshaw et al., 2004; Stentiford and Feist, 2005).

We used 2 diagnostic methods, histology and wet tissue examinations, and compared the sensitivity and specificity of histology by using the latter method as a reference test. Although we have used both methods together in parasites surveys (Eaton et al., 1991; Moran and Kent, 1999; Kent et al., 2005), we are unaware of any studies in which both methods were compared empirically with sensitivity and specificity tests. Wet preparations consistently detected more parasites. On average, only 15% of *Apophallus* sp. were correctly detected by histology (Table IV). When histology was applied to determine prevalence of gill and muscle parasites in population surveys, it had an effect on the prevalence of infection. For example, when using histologic evaluation, *Apophallus* sp. was only detected in 2 smolt populations, but it was found in 6 populations by using wet preparations. Likewise, the use of only histology would have resulted in missed *M. insidiosus* infections in smolts from 4 sites, and the prevalence of infection would have been greatly underestimated for most parasite infections except infections with gill trematodes (Tables II, III).

The sensitivity of histology improved when we excluded lightly infected fish (Table IV). However, this increase was still below 50% for all parasites examined, so less than 50% of intensely parasitized fish would be detected by histologic evaluation alone. Clearly, using wet preparations was better for detecting parasites than histology, probably because more tissue is examined. Our calculations suggest that wet tissue examination, using 1 g of muscle, represents almost 2,400 times more volume than the average amount of muscle examined in one histologic section. The estimated difference in material evaluated by both methods is similar to that reported by Kocan et al. (2011). An alternative histologic assessment, such as use of more than 1 section, or more than 1 location, within a given organ, would probably improve sensitivity.

Histology is also relatively poor for evaluating external gill parasites (Table IV) such as ciliates, because they often detach when gills are placed in fixatives; thus, data obtained by histology for these parasites are generally underestimated. For example, the reason why *C. piscium* was the only external ciliate that was detected by histology was probably because of the severity of infection by this parasite. In contrast, although *L. salmonae* infections were relatively light, this parasite was detected by both methods as this parasite is intracellular and thus would not be removed from the tissues during processing. Wet tissues also provide another advantage in that whole parasites are examined and thus important morphologic features are better visualized.

Histology, however, provides certain advantages. With the exception of the gill metacercariae, the specificity for histology was high for all other parasites (91–100%); thus, histology was equally accurate as wet preparations for identification of these parasites. Embryonated eggs of the *Sanguinicola* sp. were more often detected in histology compared with wet tissue examination (Table II; note low specificity Table IV). These eggs are deep

within blood vessels, are small and not motile, and thus are probably easier to detect by histology. Although histology had poor specificity for gill metacercariae in our analysis, this was actually due to the choice of wet preparations for the reference test. Furthermore, detection of *N. salmincola* in gills seemed to be easier by histology, which could have been due to the difficulty of detecting this worm deep in thicker wet preparations of gill.

In conclusion, we detected a wide variety of parasites in fish from some sites, and few were associated with prominent histologic changes. We found that parasites were more often detected in wet preparations. However, histology provides more information on pathology and may be more amendable for detecting parasites in certain tissues, e.g., brain of very small fish and thick tissues such as cardiac muscle. Finally, histology is a broad-based diagnostic method, allowing for detection of a variety of etiologic agents beyond parasites. We propose the use of both methods when possible to provide the most accurate identification, enumeration, and determination of impact of parasites. This is particularly important when sample availability is limited.

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