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Epizootiology and histopathology of *Parvicapsula* sp. in coho salmon *Oncorhynchus kisutch*

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ABSTRACT: The epizootiology and histopathology of the myxosporean *Parvicapsula* sp. was studied during monthly health surveys of 4 groups of coho salmon *Oncorhynchus kisutch* at a commercial farm in Puget Sound, Washington, USA, from 1984 to 1986. No *Parvicapsula* sp. was detected in histological samples taken from juvenile fish in fresh water, but the parasite was detected in fish from all groups 2 to 8 mo after transfer to seawater net pens. Groups placed in seawater net pens in November and December had a higher prevalence of infection, and a longer period of continuous detected infection, than those introduced into net pens in May. For the groups transferred to seawater in November and December, the highest infection prevalence (45 to 90%) was detected during the following March and April. Among 13 tissues examined histologically, only the pseudobranch and kidney were positive for *Parvicapsula* sp., with 26 (62%) of 42 positive fish showing infections only in the pseudobranch, 5 (12%) showing infections only in the kidney, and 11 (26%) showing infections in both organs. Both the pseudobranch and kidney were apparent primary infection sites, but pseudobranch infections appeared to persist longer in a population. Pseudobranch infections were frequently heavy and associated with extensive inflammation and necrosis of filament and lamellar tissues. The kidney had been the only infection site reported for *Parvicapsula* sp. in previous studies of coho salmon.

KEY WORDS: Kidney · Myxosporea · *Oncorhynchus kisutch* · *Parvicapsula* · Pseudobranch · Salmonid

INTRODUCTION

Myxosporeans of the genus *Parvicapsula* (family Parvicapsulidae) are coelozoic parasites in the urinary system or gall bladder, or histozoic parasites in kidneys of marine or anadromous fishes (Lom & Dyková 1992). Epizootic mortality of salmonid fishes associated with kidney infections by an unidentified *Parvicapsula* species was first observed during 1979 in juvenile coho salmon *Oncorhynchus kisutch* in seawater net pens in the state of Washington, USA (Hoffman 1984). Subsequently, *Parvicapsula* parasites of similar morphology were observed in kidneys of other species of salmonids, including chinook salmon *O. tshawytscha*, masu salmon *O. masou*, cutthroat trout *O. clarki*, and Atlantic salmon *Salmo salar*, being held in net pens near infected coho salmon in Washington state, and in Pacific cod *Gadus macrocephalus* located near the net pen site (Johnstone 1984). *Parvicapsula* sp. has also been detected in coho salmon in marine net pens in Oregon state, USA (Johnstone 1984), and in net pen-reared and wild coho salmon in British Columbia, Canada (Kent 1998). Kent et al. (1997) described a new *Parvicapsula* species, *P. minibicornis*, in kidneys of adult sockeye salmon *O. nerka* returning to a tributary of the Fraser River in British Columbia. The taxonomic relation between *P. minibicornis* and *Parvicapsula* sp. in coho salmon has not been determined.

The kidney has been the primary tissue involved in all the *Parvicapsula* infections previously reported in

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Pacific salmon *Oncorhynchus* spp. Trophozoites and developing spores of *Parvicapsula* sp. of coho salmon are observed in the epithelium and lumina of renal tubules with spores discharged from the urinary bladder (Johnstone 1984), and trophozoites may also occur in the blood and kidney interstitium, sometimes causing interstitial nephritis (Kent 1998). In contrast, *P. minibicornis* trophozoites develop in the glomeruli, and both trophozoites and spores are observed in tubular lumina, but rarely in tubular epithelium (Kent et al. 1997).

During the spring of 2002, a myxozoan identified as a *Parvicapsula* species was found in an unusual tissue location, the pseudobranch, in Atlantic salmon suffering high mortality at several seawater rearing sites in northern Norway (Karlsbakk et al. 2002, Sterud et al. 2003). This parasite has been described as a new species: *Parvicapsula pseudobranchicola* (Karlsbakk et al. 2002). The present paper reports histopathological and epizootiological aspects of earlier *Parvicapsula* sp. infections observed in pseudobranch and kidney tissues of several groups of coho salmon in seawater net pens at a fish farm in Washington state. In these fish, the pseudobranch also appeared to be a primary infection site.

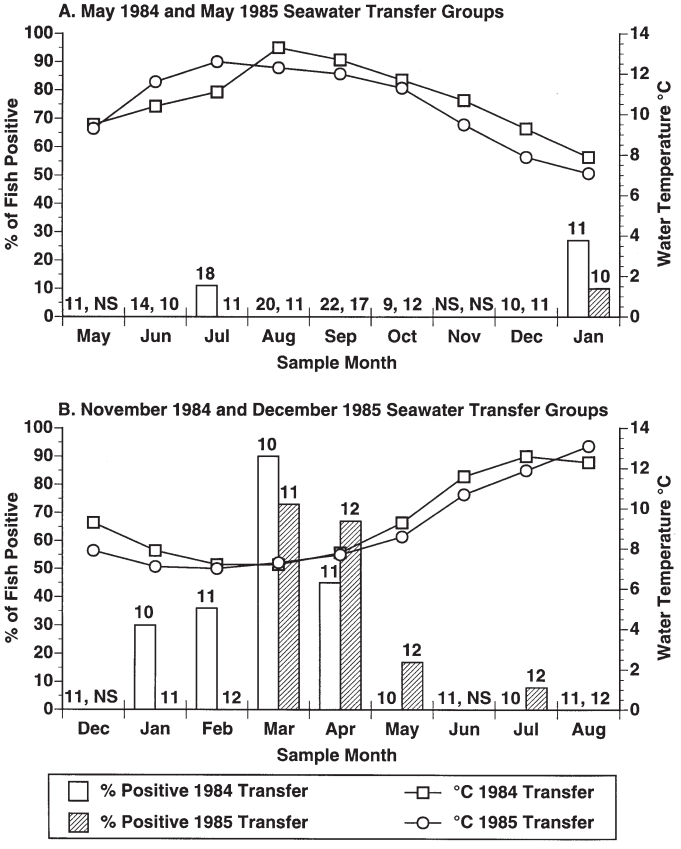


Fig. 1. Prevalence of *Parvicapsula* sp. infections in 4 groups of coho salmon *Oncorhynchus kisutch* sampled after transfer into seawater net pens in (A) spring 1984 and 1985 and (B) autumn 1984 and 1985, and mean monthly seawater temperatures during the sample periods. Prevalence data are based on detection of spores of *Parvicapsula* sp. in histological sections of kidney or pseudobranch or both. Sample sizes of fish examined histologically for *Parvicapsula* sp. are shown above the bars; NS: no sample

MATERIALS AND METHODS

As part of a fish health survey, we sampled 4 groups of juvenile coho salmon that were introduced into seawater net pens at a commercial site in Kitsap County, central Puget Sound, Washington, USA (47° 34′ N, 122° 32′ W), in May 1984, November 1984, May 1985, and December 1985 (Fig. 1). Initial samples for histological analysis were taken from each group shortly before the fish were transferred to seawater. Fish were examined monthly for *Parvicapsula* sp. and other pathogens and abnormalities after seawater introduction (with a few exceptions) until they were harvested for market ca. 9 mo after seawater introduction. During the study, seawater temperatures in the bay where the net pens were located varied from a minimum of 6.4°C in February 1986, to a maximum of 14.6°C in August 1984; mean monthly water temperatures are shown in Fig. 1.

Total numbers of fish sampled (Fig. 1) ranged from 82 (May 1985 and December 1985 seawater transfer groups) to 115 (May 1984 seawater transfer group). The gross appearance of external and internal tissues was recorded, and from each fish biopsies were made of 13 major organs or tissues, plus any additional tissues showing apparent abnormalities. Tissues sampled regularly for histopathological examination included the kidney, liver, spleen, gastrointestinal tract, pancreas, gall bladder, heart, skin, skeletal muscle, fin, gill, pseudobranch, and gonad. The tissues were fixed in 10% neutral buffered formalin, then embedded in paraffin and processed for histological examination. Sections were stained with Harris' haematoxylin and eosin, Brown and Brenn Gram stain (Luna 1968), and May-Grünwald Giemsa (Yasutake & Wales 1983).

Detection of bacterial pathogens was accomplished by observation of Gram-stained smears and histological sections of the kidney and other organs, and from cultures of kidney tissue on brain-heart infusion agar and cytophaga agar modified for marine pathogens (McDaniel 1979, Amos 1985). Biochemical and serological tests, described in the Fish Health Section/ American Fisheries Society Blue Book (McDaniel 1979,

Amos 1985), were used for presumptive and confirmatory identification of bacteria as appropriate.

RESULTS

Epizootiology

No *Parvicapsula* sp. was detected in fish sampled in fresh water, but the myxosporean was found in all 4 groups of salmon after they were transferred to salt water (Fig. 1). Infected fish in the study ranged from 13 to 29 cm in length and from 17 to 291 g in weight. *Parvicapsula* sp. infections were first detected in a given group of fish 2 to 8 mo after seawater transfer. The groups placed in seawater net pens in November 1984 and December 1985 showed a greater prevalence of infection, and a longer period of continuous detected infection, than those introduced into net pens in May 1984 and May 1985 (Fig. 1). For the groups entering seawater in November 1984 and December 1985, the highest infection prevalence (45 to 90%) was observed during March and April of the year following seawater entry. The groups transferred to seawater during May in 1984 and 1985 were harvested prior to March of the year following seawater entry, but fish positive for *Parvicapsula* sp. were detected in both groups during the final sample in January (Fig. 1). No *Parvicapsula* sp. was detected in any fish sampled during August through December of any year.

The pseudobranch had the highest rate of infection, although kidney infections were common. Of the 42 *Parvicapsula*-positive fish from which both pseudobranchs and kidneys were sampled, 26 (62%) showed infections only in the pseudobranch, 5 (12%) had infections in the kidney only, and 11 (26%) had infections in both the pseudobranch and kidney. In samples taken during the month that *Parvicapsula* sp. was first detected in a particular group of coho salmon, the parasite was usually observed in both the pseudobranch and kidney (Table 1). In subsequent months, however, *Parvicapsula* sp. was more frequently observed in the pseudobranch than in the kidney.

Table 1. Occurrence of *Parvicapsula* sp. in histological sections of the kidney and pseudobranch in coho salmon *Oncorhynchus kisutch* from which both organs were sampled. ns: no sample (fish harvested); –: no *Parvicapsula*-positive fish observed in sample

Seawater transfer date and organ	No. positive/total positive (%) Jan	Feb	Mar	Apr	May
May 1984					
Kidney	2/3 (67)	ns	ns	ns	ns
Pseudobranch	2/3 (67)	ns	ns	ns	ns
November 1984					
Kidney	2/3 (67)	2/4 (50)	0/9	0/5	–
Pseudobranch	1/3 (33)	3/4 (75)	9/9 (100)	5/5 (100)	–
May 1985					
Kidney	0/1	ns	ns	ns	ns
Pseudobranch	1/1 (100)	ns	ns	ns	ns
December 1985					
Kidney	–	–	6/8 (75)	3/7 (43)	0/2
Pseudobranch	–	–	7/8 (87)	7/7 (100)	2/2 (100)

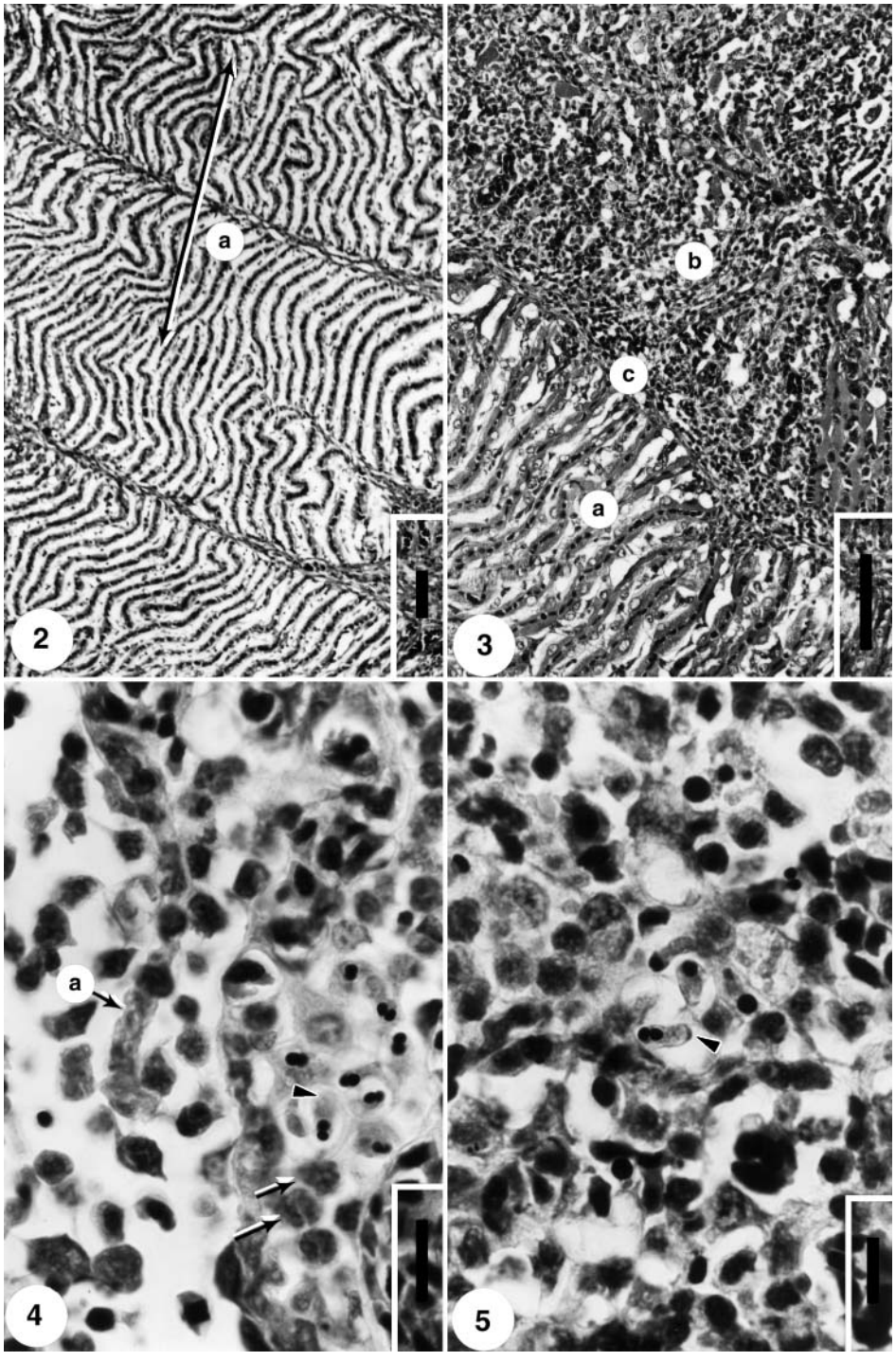
Gross pathology

In coho salmon with pseudobranch infections of *Parvicapsula* sp., all or part of the affected pseudobranch was often swollen and discoloured. The mottled tissues varied from greyish to reddish with haemorrhagic areas. Some affected areas appeared as discrete white nodules varying in size. Infected kidneys were greyish, mottled, swollen, or showed a combination of these signs. Gross signs in several fish were complicated by concurrent infections with other pathogens, including the microsporidian *Loma salmonae* (Kent et al. 1989) and the bacteria *Renibacterium salmoninarum*, *Aeromonas salmonicida* and *Vibrio* (*Listonella*) *anguillarum*.

Histopathology

Histologically, the normal coho salmon pseudobranch was observed to be separated from the external environment by an epithelial covering, and consisted of a single row of compact filaments (primary lamellae) that supported the lamellae (secondary lamellae) (Fig. 2). The lamellae and filaments were fused. Each pseudobranch lamella was supported by pillar (pilaster) cells and their flanges, forming capillaries. The epithelium of the lamellae consisted of roughly hexagonal 'pseudobranchial' cells with acidophilic cytoplasm and large ovoid nuclei showing sparse chromatin. Loose connective tissue containing elongated fibrocytes ('lacunar tissue') was usually visible between adjacent lamellae.

The pseudobranchs of *Parvicapsula*-positive fish exhibited various histopathological changes including lamellar telangiectasis, focal haemorrhages, inflammation, and necrosis. Mild to severe chronic inflammation was particularly apparent in areas of 'lacunar' connective tissue between adjacent lamellae (Figs. 3 & 4), in the connective



Figs. 2 to 5. *Oncorhynchus kisutch*. Fig. 2. Histological section through a normal pseudobranch of a juvenile coho salmon. Width of a single filament with lamellae is shown at (a). Filaments and lamellae are fused. Haematoxylin and eosin. Scale bar = 100 µm. Fig. 3. Juvenile coho salmon pseudobranch showing relatively normal lamellar tissue at lower left (a) and an area heavily infected with *Parvicapsula* sp. showing associated inflammation at upper right (b). In this section the spread of infection appears to be limited at the connective tissue core of the filament (c). Haematoxylin and eosin. Scale bar = 100 µm. Fig. 4. Juvenile coho salmon pseudobranch infected with *Parvicapsula* sp. Elongate spores (arrowhead) with 2 small spherical anterior polar capsules are visible between pseudobranch lamellae, as are trophozoite stages (arrows); some of the parasites appear to be located within the lamellar epithelium. The epithelium has completely sloughed from the lamella on the left (a), leaving the supporting pillar cells. May-Grünwald Giemsa. Scale bar = 20 µm. Fig. 5. Juvenile coho salmon pseudobranch infected with *Parvicapsula* sp. One spore shows an apparent posterior process (arrowhead). May-Grünwald Giemsa. Scale bar = 10 µm

tissue core of individual filaments (Fig. 3), and in the connective tissue at the base of the filaments. The inflammatory cell infiltrate consisted largely of mononuclear cells and lymphocytes, and proliferation of fibroblasts was common in interlamellar tissue. Necrosis was frequently observed in the infected tissue and ranged from small foci of necrotic debris to large areas of complete tissue destruction. Degeneration and necrosis of lamellar epithelial ('pseudobranchial') cells was prevalent, and in severely affected areas, much of the epithelium was sloughed from the lamellae, and only the supporting pillar cells remained (Fig. 4). Trophozoites and spores of *Parvicapsula* sp. were often numerous in areas of central and basal connective tissue of the filaments, and were particularly abundant between adjacent lamellae. The parasites sometimes appeared to be present within the lamellar epithelium (Fig. 4).

The kidneys of infected fish frequently showed numerous trophozoites and spores of *Parvicapsula* sp. in the epithelium and lumina of renal tubules, collecting ducts, and mesonephric ducts. The epithelium was often displaced by high numbers of parasites, and tubular epithelial necrosis was common. Developing spores and associated inflammation were occasionally observed in the kidney interstitium, but no *Parvicapsula* sp. were detected in the glomeruli.

Parvicapsula sp. was only identified in the pseudobranch and kidney of the coho salmon sampled. Although entities resembling *Parvicapsula* sp. trophozoites (frequently with associated foci of inflammation) were observed occasionally in histological sections of the liver, heart, spleen, pancreas and gonad, no definitive identifications were made from these organs. Detection of low numbers of *Parvicapsula* sp. that might have been present in these organs was made difficult by the presence of other pathogens in many fish.

In histological sections stained with haematoxylin and eosin, the mature spores were usually difficult to observe. However, in Gram-stained or Giemsa-stained sections (Figs. 4 & 5), the polar capsules of individual spores were readily evident. Parasites in pseudobranch and kidney sections were presumptively identified as *Parvicapsula* sp. by the elongate, asymmetrical, somewhat curved spores with 2 small spherical polar capsules at the anterior end. In histological sections, spores measured 7.5 to 8.2 × 3.3 to 4.2 µm, and polar capsules measured 1.3 to 2 µm. Some spores showed an apparent single posterior process (Fig. 5).

DISCUSSION

The coho salmon examined in this study were reared in a fish farm where *Parvicapsula* sp. had been previously diagnosed in this species (Hoffman 1984) and was associated with mortality up to 30% (Johnstone 1984). In addition, this farm was located near the marine research facility where Johnstone (1984) detected *Parvicapsula* sp. in pen-reared coho salmon as well as in other fish species. Based on a comparison of descriptions from fixed material, it is likely that the *Parvicapsula* sp. observed in our study was the same species as reported from coho salmon in the earlier studies. The sizes of mature spores in formalin-fixed sections were within the range (7 to 10 × 3 to 5 µm) reported for *Parvicapsula* sp. from coho salmon tissue sections by Kent (1992).

Our failure to detect *Parvicapsula* sp. in the juvenile coho salmon in fresh water, and observation of the parasite in fish only after 2 to 8 mo of rearing in seawater, suggested that the fish became infected in the marine environment. Johnstone (1984) similarly reported detection of the parasite in coho salmon 8 to 10 wk after the fish were introduced into seawater net pens, but did not examine fish in fresh water for the parasite.

We observed *Parvicapsula* sp. in histological samples taken from fish in the survey net pens and from fish sampled for disease diagnosis from other net pens at the seawater farm site during the months of January through July. Differences in time intervals between seawater entry and the first detection of *Parvicapsula* sp., and differences in infection prevalence among the 4 groups of coho salmon, may have reflected changes during the year in the abundance of the stage of the parasite infective for coho salmon. The higher prevalence and longer duration of infection in the groups transferred to seawater in November and December, compared with those transferred in May, suggested that the infective stage was most abundant from late autumn through late winter, based on the shortest time between seawater transfer and definitive identification of *Parvicapsula* sp. in fish in this study (ca. 8 wk). The detection of *Parvicapsula* sp. in both May seawater transfer groups in the sample taken the following January, although the parasite had not been detected in either group for at least 5 mo prior to this, also supports this hypothesis. Because the May seawater transfer groups were harvested shortly after the January samples were taken, it was not possible to observe whether the infections in these groups would have continued for several months in a pattern similar to the November and December seawater transfer groups.

The precise incubation period for *Parvicapsula* sp. in naturally infected fish is unknown. In laboratory studies, however, Johnstone (1984) reported detection of early trophozoite stages after 3 to 5 wk and spore stages after 8 to 10 wk in kidneys of coho salmon following exposure of fish by gastric intubation to kidney homogenates from naturally infected fish. Similarly,

we observed spores of *Parvicapsula* sp. in the kidneys or pseudobranchs of fish a minimum of 8 wk after seawater entry.

The source of the *Parvicapsula* sp. in the coho salmon was not determined. Johnstone (1984) suggested that Pacific cod *Gadus macrocephalus* might be a natural reservoir of infection, because she observed spores morphologically similar to *Parvicapsula* sp. in kidneys of Pacific cod sampled near the salmon net pens. Johnstone's reported success with a laboratory infection of coho salmon that were transferred from a freshwater hatchery and held in tanks of artificial seawater during the experiment also suggests the potential of a direct life-cycle for this parasite. Direct fish-to-fish transmission of the myxozoan *Myxidium leei* by cohabitation, effluent exposure, or feeding of infected fish tissues has been reported in laboratory studies with sea bream *Sparus aurata* (Diamant 1997), but the possibility exists that these experiments demonstrated transfer of infection by trophozoites rather than transmission by mature myxospores (Kent et al. 2001). The myxozoan species for which the life cycles have been most fully elucidated require both a fish host and an invertebrate host (an oligochaete, polychaete, or bryozoan) for completion of the complex life cycles (Kent et al. 2001). Johnstone (1984) examined Gram-stained smears of internal organs from 1 polychaete species (*Nereis vexillosa*) and 10 crustacean species that inhabited the salmon net pens and did not detect *Parvicapsula* sp. However, she likely examined the smears for typical salmonid-type *Parvicapsula* sp. spores rather than the actinosporean spores that might be present in an invertebrate host.

Although *Parvicapsula* sp. had been observed since 1979 in kidneys of coho salmon from net pens in the area of Puget Sound where our study was conducted, the parasite had not been previously reported from the pseudobranch of this species. It is possible that the pseudobranchs of coho salmon were not infected with *Parvicapsula* sp. during the earlier epizootics, but it is also conceivable that the parasite was not observed in the pseudobranch because this organ was not routinely examined and sampled as it was in our study. Because the normal pseudobranch of coho salmon, similar to that of rainbow trout (Mattey et al. 1978, Yasutake & Wales 1983, Laurent & Dunel-Erb 1984), is a small, inconspicuous, epithelium-covered hemibranch on the inner surface of the operculum, it is easy to overlook during routine diagnostic examination. Finally, the possibility that the parasite observed in the pseudobranch in the present study is a different species from the *Parvicapsula* sp. detected in the kidney in earlier studies cannot be dismissed without further investigation by molecular biological methods.

In contrast to pseudobranch infections by *Parvicapsula pseudobranchicola* recently described in farmed Atlantic salmon in Norway (Karlsbakk et al. 2002, Sterud et al. 2003), which were usually associated with conspicuous haemorrhages in the eyes, pseudobranch infections of *Parvicapsula* sp. in coho salmon were rarely associated with grossly visible eye abnormalities. Among the 37 fish with pseudobranch infections of *Parvicapsula* sp. in our study, only 5 showed eye abnormalities, with 3 fish showing exophthalmos and 2 showing lesions indicative of eye trauma. In 2 fish, the exophthalmos was associated with *Renibacterium salmoninarum* infections in postorbital tissues. *Parvicapsula* sp. infections in coho salmon also appeared to result in less consistent anaemia than those reported in Atlantic salmon. Whereas mean haematocrit values in *Parvicapsula*-infected Atlantic salmon in 2 farms were 26 and 22% (Sterud et al. 2003), the mean haematocrit value was 35% (range 6 to 58%) among *Parvicapsula*-infected coho salmon in our study (excluding fish with concurrent systemic infections with other pathogens such as *Renibacterium salmoninarum*).

Our finding of *Parvicapsula* sp. in both the pseudobranch and kidney of the majority of infected coho salmon at the time of initial detection suggested that both organs were primary infection sites. However, the parasite appeared to persist longer in the pseudobranch in a given net pen population. Thus, the detection of *Parvicapsula* sp. in either or both organs might depend on the stage of infection at which fish are sampled. Johnstone (1984) reported that the parasite persisted in a net pen population of coho salmon for 5 wk based on kidney samples only, whereas we observed persistence of the parasite in a population for at least 4 mo by analysis of samples from both kidney and pseudobranch tissues.

Histopathological changes associated with *Parvicapsula* sp. infections in the kidney of coho salmon were similar to those reported by other investigators (Hoffman 1984, Johnstone 1984, Kent et al. 1997, Kent 1998). Heavy infections of *Parvicapsula* sp. in both the kidney and pseudobranch were associated with tissue destruction. A more consistent and severe host inflammatory response was generally observed in pseudobranch infections.

Our observation of numerous trophozoites and spores of *Parvicapsula* sp. between pseudobranch lamellae was similar to the findings of Karlsbakk et al. (2002) and Sterud et al. (2003), who observed trophozoites and spores of *Parvicapsula* massed in distended spaces between pseudobranchial lamellar capillaries of Atlantic salmon. Nevertheless, the results of analyses of multiple Atlantic salmon tissues for *Parvicapsula* differed from the results of our investigation of coho salmon tissues. Karlsbakk et al. (2002) only detected

P. pseudobranchicola in the pseudobranchs among tissues they examined by squash preparation (pseudobranch, gill, kidney, spleen, gall bladder, urinary bladder, medulla oblongata, and medulla spinalis). Sterud et al. (2003) detected the parasite in the pseudobranchs of all 40 fish examined (in wet mounts, in histological sections, or both), but also detected spores and pseudoplasmodia in the gills, liver, and kidney interstitium of 7, 6, and 1 fish, respectively.

In the present study no measurements were made from fresh spores for comparison with other *Parvicapsula* species, and no fresh material has been available since the coho salmon net pen farms ceased operation in areas of Washington state where the parasite was common. Nevertheless, we concur with the belief of Kent et al. (1997) that this *Parvicapsula* sp. in coho salmon is likely a different species than *P. minibicornis* in sockeye salmon. *P. minibicornis* develops in kidney glomeruli and tubular lumina (Kent et al. 1997), whereas *Parvicapsula* sp. develops in both the epithelium and lumina of the tubules but not in the glomeruli. In addition, the spores of *P. minibicornis* are symmetrical with pyriform polar capsules and 2 posterior appendages (Kent et al. 1997), whereas spores of *Parvicapsula* sp. observed in this and other studies (Hoffman 1984, Johnstone 1984) are asymmetrical with spherical polar capsules and a single posterior process. Preliminary morphological comparisons of *P. pseudobranchicola* from Atlantic salmon in Norway indicate that the Atlantic salmon parasite is more similar to the coho salmon *Parvicapsula* sp. than to *P. minibicornis* (Sterud et al. 2003). However, Karlsbakk et al. (2002) observed apparent differences between *P. pseudobranchicola* and the coho salmon *Parvicapsula* sp. described by Hoffman (1984) in the positioning of the suture and polar capsules of the spore. Critical morphological evaluations of spores and rDNA sequence comparisons are needed to resolve the relationships among the coho salmon *Parvicapsula* sp., *P. pseudobranchicola*, and *P. minibicornis*.

With the decline of coho salmon net pen culture in the Pacific Northwest region of the USA and Canada, interest in the *Parvicapsula* sp. found in these fish also diminished. However, recent reports of a similar *Parvicapsula* species in Atlantic salmon has sparked renewed interest in the coho salmon parasite. It is hoped that this previous study of *Parvicapsula* sp. in coho salmon can provide information useful for investigations of the recent occurrence of Atlantic salmon parvicapsulosis.

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