Vol. 106: 207–215, 2013
doi: 10.3354/dao02657

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published November 6

Impact of *Ichthyophonus* infection on spawning success of Yukon River Chinook salmon *Oncorhynchus tshawytscha*

Toshihide Hamazaki[1,*], Eryn Kahler[1], Bonnie M. Borba[2], Tamara Burton[1]

[1]Alaska Department of Fish and Game, Division of Commercial Fisheries, 333 Raspberry Road, Anchorage, Alaska 99518, USA
[2]Alaska Department of Fish and Game, Division of Commercial Fisheries, 1300 College Road, Fairbanks, Alaska 99701, USA

ABSTRACT: We examined the impacts of *Ichthyophonus* infection on spawning success of Yukon River Chinook salmon *Oncorhynchus tshawytscha* at spawning grounds of the Chena and Salcha Rivers, Alaska, USA. During the period 2005 to 2006, 1281 salmon carcasses (628 male, 652 female) were collected throughout the spawning season and from the entire spawning reaches of the Chena and Salcha Rivers. For each fish, infection status was determined by culture method and visual inspection of lesions of heart tissue as uninfected (culture negative), infected without lesions (culture positive with no visible lesions), and infected with lesions (culture positive with visible lesions), and spawning status was determined by visually inspecting the percentage of gametes remaining as full-spawned (<10 %), partial-spawned (10–50 %), and unspawned (>50 %). Among the 3 groups, the proportion of full-spawned (i.e. spawning success) females was lower for those infected without lesions (69 %) than those uninfected (87 %) and infected with lesions (86 %), but this did not apply to males (uninfected 42 %, infected without lesions 38 %, infected with lesions 41 %). At the population level, the combined (infected and uninfected) proportion of female spawning success was 86 %, compared to 87 % when all females were assumed uninfected. These data suggest that while *Ichthyophonus* infection slightly reduces spawning success of infected females, its impact on the spawning population as a whole appears minimal.

KEY WORDS: Ichthyophoniasis · Protozoan · Parasite · Breeding success · Chena River · Salcha River

INTRODUCTION

In evaluating the impacts of pathogens and diseases on wild salmon populations, many studies have focused on prevalence and lethal effects during their spawning migration (e.g. % of fish killed by diseases or pathogens during migration); however, few studies have focused on sub-lethal effects, such as the ability of the infected fish at spawning grounds to reproduce successfully (Kent 2011, Stephen et al. 2011). Many fish at the spawning grounds are infected with various pathogens (Cramer et al. 2002, Mann et al. 2010, Loch et al. 2012), and some fish, especially highly stressed individuals, may fail to spawn successfully (Quinn et al. 2007, Keefer et al. 2008, Young & Blenden 2011). Because immunological functions of spawning salmon are reduced (Brett 1995, Maule et al. 1996, Miller et al. 2009, 2011, Magnadóttir 2010), the infected fish may fail to spawn. Thus, ignoring the sub-lethal effects could underestimate potential impacts of disease/pathogens on the host population.

Absence of studies on sub-lethal effects is also the case for *Ichthyophonus hoferi* (hereafter referred to as *Ichthyophonus* because of taxonomic uncertainties of strains) infections in Yukon River Chinook

*Email: toshihide.hamazaki@alaska.gov

salmon *Oncorhynchus tshawytscha*. *Ichthyophonus* is a protozoan parasite of marine and anadromous fishes, such as rockfish (Sebastidae), Pacific halibut *Hippoglossus stenolepis*, herring (*Clupea* spp.), and salmons (*Oncorhynchus* spp.) (McVicar 1999, Meyers et al. 2007). Some fishes (e.g. rockfish, halibut) may live normally while infected, but *Ichthyophonus* is also known to cause outbreaks and mass mortality of Atlantic and Pacific herring (Patterson 1996, Mellergaard & Spanggaard 1997, Kramer-Schadt et al. 2010). Thus far, mass mortality of salmon species by *Ichthyophonus* infection has not been reported.

In the Yukon River, *Ichthyophonus* infection in Chinook salmon was first observed in the late 1980s (ADFG 1988). After that, no incidence of *Ichthyophonus* infection was reported until the late 1990s, when the infection became widely noticeable throughout the river drainage. The source of *Ichthyophnus* infection for Yukon River Chinook salmon is unknown, but it is believed that infection occurs in the ocean prior to freshwater migration through ingestion of infected prey (Criscione et al. 2002, Jones & Dawe 2002, Kocan et al. 2004a). *Ichthyophonus* primarily infects cardiac muscles but spreads throughout the body of a host, which reduces swimming performance and stamina of the host, and leads to its mortality (Tierney & Farrell 2004, Kocan et al. 2006, 2011). During the period 1999 to 2003, *Ichthyophonus* infection prevalence in the Yukon River was about 25% at the river mouth, peaked at 35% in mid-river, and was about 10% at the spawning grounds (Kocan et al. 2004a). This decline from mid-river to spawning grounds was attributed to mortality of the infected fish, in which about 60% of the infected fish die en route to the spawning grounds (Kocan et al. 2004a). Conversely, this implies that about 40% of infected salmon reach the spawning grounds. Thus far, spawning success of infected fish has not been examined.

Very few studies have examined the impacts of pathogens on spawning salmon *in situ* (e.g. Mann et al. 2010). Part of the difficulty is that all salmon die at spawning grounds regardless of their infection or spawning status. Many salmon at spawning grounds are also infected with multiple pathogens that are potentially lethal (Mann et al. 2010, Loch et al. 2012). These confounding conditions make it difficult to attribute spawning failure to a particular pathogen. Spawning failure of the infected fish may be caused by (1) pathogens directly (e.g. pathogen load reaches lethal levels), (2) pathogens indirectly (e.g. infected fish are too burdened by pathogens to build redds, mate, and spawn), or (3) other factors (e.g. high temperature, high density, high stresses caused by factors other than parasite infection), while the presence and severity of pathogens are coincidental. For instance, even though Mann et al. (2010) found that spring Chinook salmon of the Willamette River (Oregon, USA) that died without spawning were more severely infected by freshwater parasites (*Nanophyetus salminicola, Apophallus* sp., *Echinochasmus milivi, Parvicapsula minibicornis*), they were not able to attribute the spawning failure to those parasites because (1) infection severity of those fish was below the lethal level (i.e. the fish were not killed by the pathogens directly), (2) some spawned fish were more severely infected than those that died without spawning (i.e. severity of infection may not be related to spawning failure), and (3) severity of infection was associated with other factors, such as timing of arrival at the spawning grounds (i.e. infection severity may be coincidental).

In contrast, circumstances surrounding *Ichthyophonus* infection on Yukon River Chinook salmon makes it possible to attribute spawning failure to *Ichthyophonus*. First, in the Yukon River no pathogens other than *Ichthyophonus* are known to cause significant mortality at spawning grounds. Second, *Ichthyophonus* infection occurs only in the marine environment, so that uninfected fish remain free of *Ichthyophonus* during the freshwater migration stage. During spawning migration stage, *Ichthyophonus*-infected fish are more burdened than uninfected fish. This makes it possible to attribute differences in spawning failure between infected and uninfected fish to *Ichthyophonus* infection. Here, we examined the impacts of *Ichthyophonus* infection on spawning success of the Yukon River Chinook salmon at spawning grounds of the Chena and Salcha Rivers. We evaluated the impacts both at the individual level (e.g. do infected fish spawn as successfully as uninfected fish?) and at the population level (e.g. does *Ichthyophonus* infection reduce overall spawning success of a population?).

MATERIALS AND METHODS

Sampling locations

The Chena and Salcha Rivers are major spawning grounds of the Chinook salmon Tanana River stock (Eiler et al. 2004, 2006a,b). They are north bank tributaries of the Tanana River, 1472 and 1544 river km (rkm), respectively, from the southern mouth of the Yukon River (Fig. 1). Escapement of Chinook salmon on these rivers is monitored at salmon escapement

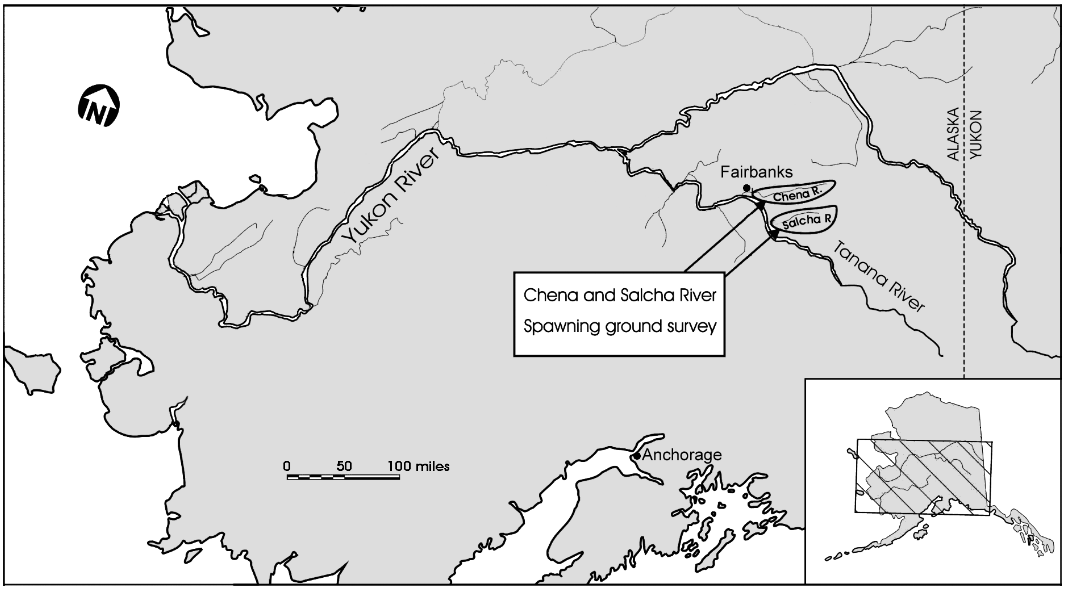

Fig. 1. Yukon River and sampling area in Alaska, USA

counting tower sites located 76 rkm (Chena) and 5 rkm (Salcha), respectively, upriver from their confluences (Fig. 2, Brase 2012). From the tower sites, these rivers extend 160 rkm (Chena) and 200 rkm (Salcha), where salmon spawn throughout the entire river reaches (Fig. 2). During the period 2005 to 2006 Chinook salmon carcasses were collected every 2 to 3 d throughout the season (mid- to late August) alternately from the lower (0–50 rkm) or upper (50–200 rkm) reaches (Fig. 2, Table 1, Kahler et al. 2007, 2011). Sampling of carcasses was limited to those meeting the following criteria: clear eyes, red-pink colored gills, and firm heart. These fish were considered to have died within the previous 24 h (Kocan et al. 2004a, Kahler et al. 2011).

Tissue sample collection

From each fish, about 0.5 g of heart muscle tissue was sampled at the riverbank, using an aseptic technique avoiding any cross contamination (Kahler et al. 2007, 2011). The tissue samples were stored in a refrigerated *Ichthyophonus* culture medium and were shipped to the Alaska Department of Fish and Game (ADFG) pathology laboratory in Anchorage within 2 to 3 d. The fish were also examined for the presence of white spots on the heart, kidney, and spleen, which might be indicative of *Ichthyophonus* infection. Spawning status of females was determined by a visual comparison of the remaining egg volume relative to the size of the visceral cavity, clas-

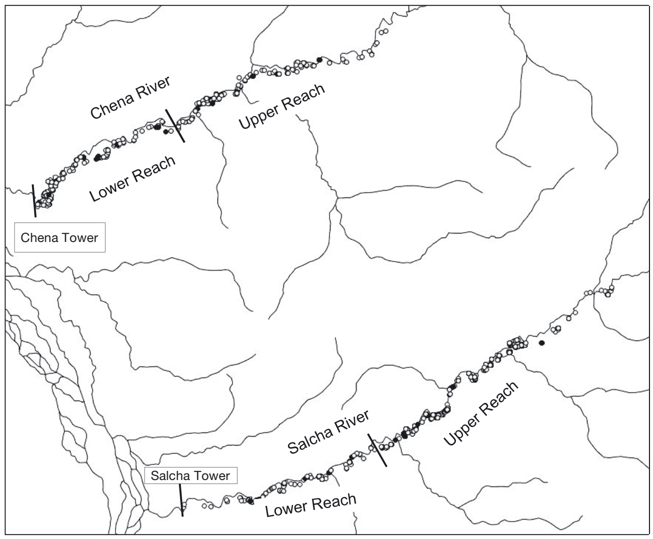

Fig. 2. *Oncorhynchus tshawytscha.* Chena and Salcha River drainages showing salmon escapement counting tower locations and locations of carcasses. Black circles indicate infected fish and white circles indicate uninfected fish

Table 1. *Oncorhynchus tshawytscha.* Number of Yukon River Chinook salmon sampled and successfully tested for *Ichthyophonus* infection. In 2006, the sampling target was reduced to about half that of 2005. rkm: river km

Location	rkm	Date sampled	Year	Male	Female
Chena	1472	22 Jul–11 Aug	2005	170	147
River		28 Jul–8 Aug	2006	73	84
Salcha	1544	17 Jul–8 Aug	2005	253	311
River		28 Jul–10 Aug	2006	132	111

sified as full-spawned (<10% of the eggs remained), partial-spawned (10–50% of the eggs remained), or unspawned (>50% of the eggs remained). Similarly, spawning status of males was assessed based on a visual comparison of the remaining sperm volume and testes. We considered only full-spawned males as 'successful spawners.' This criterion was also used by Cramer et al. (2002) and Hamstreet (2009, 2011), but is stricter than that used by Mann et al. (2010) and Young & Blenden (2011) (<25% of gametes remaining).

Diagnosis of *Ichthyophonus* infection

Infection of *Ichthyophonus* was diagnosed by the explant culture method (McVicar 1999). The tissue was cultured in 7 ml Eagle's minimal essential medium supplemented with 5% fetal bovine serum, 100 IU ml^{-1} penicillin, 100 µg ml^{-1} streptomycin, and 100 µg ml^{-1} gentamicin (*Ichthyophonus* culture medium) and incubated at 14°C for a minimum of 14 d. The cultures were periodically examined microscopically for the presence of *Ichthyophonus*. Based on presence or absence of *Ichthyophonus* in the culture samples, each fish was diagnosed as either uninfected (absent) or infected (present). Of those diagnosed as infected, fish with white lesions/spots on the heart (i.e. typical observable sign of icthyophoniasis) were classified as 'infected with lesions' and those without lesions were classified as 'infected without lesions.' The above classification corresponds to the classification of 'clinical' and 'subclinical' used inappropriately by Kocan et al. (2004b, 2011) (because 'clinical' signs of desease pertain to abnormal signs and symptons observable in a living organism). In a previous study, about 80% of fish with visible lesions on the heart were culture positive (n = 191), and infected fish with visible lesions had an average histological intensity of 3.3 (no. of *Ichthyophonus* per 10× microscopic field) (n = 22), whereas it was 0.8 for infected fish without lesions (n = 6; ADFG unpubl. data). Simultaneously, of the 22 infected fish with lesions, 6 fish (27%) had 0 histological intensity. This suggests that some lesions (including those found in *Ichthyophonus* infected fish) can be caused by other fish pathogens (Fish 1939, Finn & Nielson 1971, Corbel 1975, Dykova & Lom 1978) and that histology may miss the presence of, and underestimate severity of, *Ichthyophonus* infection (Kocan et al. 2011, Kent et al. 2013). In this study, we assumed that the majority of lesions found were caused by *Ichthyophonus* and that infected fish with lesions were more severely infected than infected fish without lesions.

Data analyses

We separated analyses between females and males and combined all usable data (i.e. Chena and Salcha Rivers, all years) to increase analytical power. Infection prevalence ($p_i = n_i/[n_i + n_u]$, where n_i and n_u are the number of infected and uninfected fish, respectively) was compared among age groups (ages 4, 5, 6, and 7 for males; ages 5, 6, and 7 for females), between early (July) and late (August) timing, and between the lower (0–50 rkm) and the upper (50–200 rkm) reaches of the spawning grounds. For comparison among ages, a chi-square test was used, and the 2-sided z-test was used for other comparisons. We defined 'spawning success' as full-spawned fish, and compared its proportion between infected ($p_{s,i} = n_{s,i}/n_i$) and uninfected fish ($p_{s,u} = n_{s,u}/n_u$), where $n_{s,i}$ and $n_{s,u}$ are the number of full-spawned infected and uninfected fish. In all above analyses, a critical value of $\alpha = 0.05$ was used for determination of statistical significance. At the population level, the impacts of *Ichthyophonus* were evaluated by calculating overall proportion of spawning success as $p_{s,t} = (n_{s,i} + n_{s,u})/(n_i + n_u)$, or $p_{s,t} = p_{s,u} - p_i\,(p_{s,u} - p_{s,i})$. This equation indicates that impacts of *Ichthyophonus* infection on overall spawning success are influenced by (1) differences in spawning success between uninfected and infected fish ($p_{s,u} - p_{s,i}$), and (2) infection prevalence at the spawning ground (p_i). If infected fish had lower spawning sucess than uninfected fish, and infection prevalence is high, the impacts of *Ichthyophonus* on the population level spawning success would become high.

RESULTS

Characteristics of *Ichthyophonus* infection at Chena and Salcha spawning grounds

Infection prevalence was similar at the Chena and Salcha spawning grounds (Table 2). Among ages, both females and males showed higher infection prevalence in older fish (Fig. 3), which was significant for females ($\chi^2 = 19.4$, df = 2, p = 0.00006), but not significant for males ($\chi^2 = 3.3$, df = 3, p = 0.345). Within the spawning ground, the prevalence appeared higher at the lower (<50 rkm) reach than the upper (50–200 rkm) reach for both females and males across years and rivers (Table 2). The difference was significant for females ($z = 2.105$, p = 0.035), but was not significant for males ($z = 1.127$, p = 0.260). Among the infected fish, 59% of females and 65% of males

Table 2. *Oncorhynchus tshawytscha.* Prevalence of *Ichthyophonus* infection (± 95%CI) for males and females for each river, year, and spawning reach

	Year	Reach	Male	Female
Chena River	2005	Lower	10.0 ± 5.9	15.6 ± 7.3
		Upper	11.4 ± 7.5	3.9 ± 5.4
	2006	Lower	18.4 ± 12.5	9.3 ± 8.7
		Upper	14.3 ± 11.8	9.8 ± 9.2
Salcha River	2005	Lower	9.6 ± 4.6	13.3 ± 5.2
		Upper	8.2 ± 5.5	7.6 ± 4.3
	2006	Lower	14.5 ± 8.0	12.3 ± 8.1
		Upper	7.1 ± 6.8	10.9 ± 9.1
Total		Lower	11.6 ± 3.3	13.2 ± 3.5
		Upper	9.7 ± 3.6	7.8 ± 3.1
		Total	10.8 ± 2.4	10.9 ± 2.4

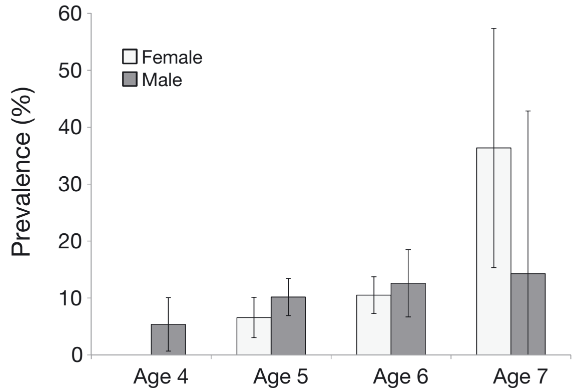

Fig. 3. *Oncorhynchus tshawytscha. Ichthyophonus* infection prevalence (95% CI range) by age (yr)

had lesions, and the proportion appeared higher in the upper than the lower reaches for both females (upper: 64%, lower 57%) and males (upper: 76%, lower 64%); however the differences were not significant for either sex. During the spawning periods, fish that arrived in July appeared to have lower infection prevalence than those that arrived in August for both females (July: 9.4%, August 11.8%) and males (July: 8.0%, August 12.1%); however, these differences were not significant. Further, of the infected fish, individuals that arrived in July appeared more infected with lesions than those that arrived in August for both females (July: 65%, August: 57%) and males (July: 81%, August: 60%); however, the differences were not significant.

Overall infection prevalence was 10.8% for females and 10.9% for males (Table 2).

Spawning success

The proportion of full-spawned individuals (spawning success) was consistently over 70% for females, but varied from 13 to 61% for males (Table 3). Across years and rivers, infected fish showed consistently lower spawning success than uninfected fish for females, whereas for males, infected fish had higher spawning success except for Salcha in 2005 (Table 3). The difference, however, was not significant for either females (z-test, z = 1.90, p = 0.058) or males (z = 0.30, p = 0.764). Of those infected, fish with lesions showed higher spawning success than fish without lesions for both females (without lesions: 69%, n = 29; with lesions: 86%, n = 42) and males (without lesions: 38%, n = 24; with lesions: 41%, n = 44; Fig. 4). Among the 3 groups, infected fish without lesions had the lowest spawning success for females (χ^2 = 7.64, df = 2, p = 0.022), but not for males (χ^2 = 0.16, df = 2, p = 0.921). Within spawning grounds, females had higher spawning success at the upper reach than those at the lower reach for both uninfected (lower 83.5%, upper 91.5%) and infected fish (lower 77.6%, upper 81.8%). However, the difference was significant only for uninfected (z = 2.88, p = 0.004) but not for infected fish (z = 0.407, p = 0.68). In

Table 3. *Oncorhynchus tshawytscha.* Comparison (%) of full-spawned, partial-spawned, and unspawned fish between uninfected fish and those infected with *Ichthyophonus*, and overall spawning success (%), 2005 to 2006

	Uninfected				Infected				Overall
	n	Full	Partial	Un-spawned	n	Full	Partial	Un-spawned	spawning success
Females									
Chena									
2005	130	85.5	12.1	2.4	17	76.5	17.6	5.9	85.0
2006	76	92.1	5.3	2.6	8	75.0	25.0	0.0	83.3
Salcha									
2005	278	82.7	12.9	4.0	33	81.8	18.2	0.0	82.9
2006	98	95.9	4.1	0.0	13	76.9	15.4	7.7	84.7
Total	582	87.0	10.3	2.8	71	78.9	18.3	2.8	86.2
Males									
Chena									
2005	152	59.2	34.9	5.9	18	61.1	33.3	5.6	59.4
2006	61	13.1	70.5	16.4	12	50.0	41.7	8.3	19.2
Salcha									
2005	230	38.3	26.1	35.7	23	13.0	30.4	56.5	36.0
2006	117	40.2	33.3	26.5	15	46.7	20.0	33.3	40.9
Total	560	41.8	34.4	23.8	68	39.7	30.9	29.4	41.4

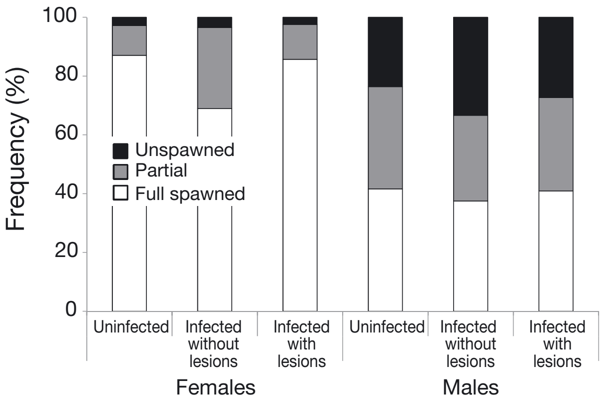

Fig. 4. *Oncorhynchus tshawytscha.* Spawning condition of females and males that were uninfected by *Ichthyophonus*, infected without lesions, and infected with lesions

contrast, males had lower spawning success at the upper reach than at the lower reach for uninfected fish (lower 43.7%, upper 38.6%), whereas infected males spawned equally (lower 39.5%, upper 40.0%). The difference was not significant for either uninfected (z = 1.208, p = 0.227) or infected fish (z = 0.038, p = 0.970).

At the population level, the overall spawning success rate ($p_{s,t}$) of Chena and Salcha River Chinook salmon was similar to that of uninfected fish ($p_{s,u}$) for both years and sex (Table 3). The overall spawning success was higher than that of uninfected fish for males in 2006 because spawning success of infected males was higher than that of uninfected males (Table 3). For each river and year, the overall spawning success rate ranged from 82.6 to 93.7% for females and from 19.2 to 59.4% for males (Table 3).

DISCUSSION

This study is the first to examine the impacts of a pathogen on spawning success of Chinook salmon *in situ*, at both the individual level (e.g. does *Ichthyophonus* reduce spawning ability of the infected fish?) and population level (e.g. does *Ichthyophonus* reduce reproductive capacity of the infected fish population?). The major finding was that although *Ichthyophonus*-infected females showed lower spawning success than uninfected females, the difference was not statistically significant or only marginal at α = 0.05. At the population level, the overall spawning success rate was similar to that of uninfected fish (Table 3). These data suggest that the impact of *Ichthyophonus* infection on spawners at spawning grounds is minimal at both individual and population levels.

Among our findings, the most puzzling was a significantly lower spawning success of infected fish without lesions, or that infected fish with lesions spawned as successfully as uninfected fish. Further, more infected fish with lesions spawned at the upper reaches of the spawning ground. Since this was consistently observed between years and spawning rivers, a sampling anomaly is unlikely. The finding is contrary to our understanding of cumulative impacts of infection on hosts, that more severely infected fish (e.g. with lesions) should perform more poorly than less severely infected (e.g. without lesions) and uninfected fish (Kent 2011). One explanation is that the difference in infection severity between fish with and without lesions is small at the spawning grounds because more severely infected fish have died on the way there. In fact, the proportion of severely infected fish declined from lower river to upriver sites during the spawning migration (Kocan et al. 2004a,b, 2006). At the Chena River spawning ground, histological intensity of infected fish with lesions was less than 1 per 10× microscopic field (n = 4) (Kocan et al. 2004). Furthermore, the lesions in the infected fish could be generated by other pathogens. However, those do not explain underperformance of only infected fish without lesions. More comprehensive surveys are needed to understand underperformance of the infected fish without lesions.

At the population level, the negligible impacts of *Ichthyophonus* infection on overall spawning success was due to both (1) a marginal difference in spawning success between infected and uninfected fish ($p_{s,u}$ – $p_{s,i}$ = 0.081 on average) and (2) low prevalence at the spawning ground (p_i = 0.109 on average), which resulted in overall impact of 0.009 or 1% of difference in spawning success between uninfected fish and overall. Simultaneously, we acknowledge that our definition of 'spawning success' as expulsions of gametes does not necessarily mean that fish spawn successfully. For instance, infected fish may expel eggs prematurely, may not be able to build and defend redds as successfully as the uninfected fish, or their eggs may not be as viable as those from uninfected progenitors. At the population level, a more desirable evaluation measure would be 'reproductive/breeding success,' or survival of progeny from infected progenitors to reproductive adulthood. Thus far, impacts of infection on host spawning behavior and viability of offspring have not been well studied. Vertical transmission of parasites from infected females to their eggs is rare (Magnadóttir 2010), and no case has been reported regarding *Ichthyophonus*. Considering that the sub-lethal impact of infection is increased stress level, more highly stressed females tend to produce fewer and lower-weight eggs (Contreras-Sánchez et al. 1998, Schreck et al. 2001, Ramsay et al. 2009). Juveniles from highly stressed females also survived at lower rates than those from unstressed females (Eriksen et al. 2007, Gagliano & McCormick 2009); however, those from moderately stressed females survived at a rate similar to those from unstressed females (Contreras-Sánchez et al. 1998). The effects of stress on offspring survival depend on the level of stress. The difference in stress levels from *Ichthyophonus* between infected and uninfected females at the spawning ground is unknown. The worst-case scenario would be assuming that all offspring from infected females are unviable or that all infected females would fail to spawn ($p_{s,i}$ = 0), and the overall spawning success would be 77 %, or 10 % different from that of uninfected fish (87 %). This decline can be considered great if it is beyond the range of natural variation of spawning success; however, long-term spawning success data do not exist for the Chena and Salcha Rivers. In other rivers where impacts of parasite/disease are considered negligible, annual spawning success rate ranged from 70 to 100 % (e.g. Hamstreet 2009, 2011, Young & Blenden 2011). This suggests that the worst-case scenario impact of *Ichthyophonus* infection is still within the range of natural variation.

Thus far, we focused evaluation of the impacts of *Ichthyophonus* infection primarily on females, partially because the data suggest minimal impacts of *Ichthyophonus* infection on males. Sex differences in the impacts of *Ichthyophonus* infection have been reported, but the results are not consistent. For instance, *Ichthyophonus* infection prevalence was lower in males than in females for Chinook salmon (Kocan et al. 2004a,b) and rockfish (Halos et al. 2005), but was higher for sea bass *Dicentrarchus labrax* (Sitja-Bobadilla & Alvarez-Pellitero 1990) and brown trout *Salmo trutta* (Schmidt-Posthaus & Wahli 2002). The difference in prevalence can be attributed to exposure (e.g. females feed on more infected prey than males) and to susceptibility (e.g. females are more susceptible to infection than males), but separating the two is difficult *in situ*. Artificial infections with *Ichthyophonus* in the laboratory have been conducted in various studies (e.g. Jones & Dawe 2002, Kocan et al. 2006, 2009, 2011, El-Ghany & El-Ashram 2008), but we found only one study comparing a difference in infection susceptibility between males and females, in which tilapia *Oreochromis niloticus* females had higher *Ichthyophonus* infection prevalence than males (El-Ghany & El-Ashram 2008). As for potential impacts of *Ichthyophonus* on reproductive success of males, few studies have been conducted on male spawning of Chinook salmon *in situ* (e.g. Berejikian et al. 2010). Further studies are needed regarding infection effects on males, such as mating success of infected males and viability of sperm and progeny of infected males.

In conclusion, our study suggests that impacts of *Ichthyophonus* infection on spawning success of Chinook salmon at the spawning ground are slight to minimal at both individual and population levels. However, empirical evaluations of assumptions are needed to fully evaluate population level impacts. Finally, as our study is the first to compare spawning success of infected fish to that of uninfected fish at the spawning ground, further studies are imperative for generalization of our results.

Acknowledgements. Primary funding for this research was provided to the Alaska Department of Fish and Game by the Pacific Salmon Recovery Fund. Additional support was provided by the National Marine Fisheries Service, the Bering Sea Fishermen's Association, the US Geological Survey, and the US Fish and Wildlife Service. We thank all field crews for sample collections. We thank D. Crawford, E. Volk, and J. Ferguson for critical reviews and editorial corrections of the manuscript.

LITERATURE CITED

ADFG (Alaska Department of Fish & Game) (1988) Fish pathology report 890026. ADFG, Fish Pathology Section, Anchorage, AK

Berejikian BA, Van Doornik DM, Endicott RC, Hoffnagle TL, Tezak EP, Moore ME, Atkins J (2010) Mating success of alternative male phenotypes and evidence for frequency-dependent selection in Chinook salmon, *Oncorhynchus tshawytscha.* Can J Fish Aquat Sci 67:1933–1941

Brase ALJ (2012) Chinook and coho salmon escapement in the Chena, Delta Clearwater, Goodpaster and Salcha River. Fishery Data Series No. 12-01. Alaska Department of Fish & Game, Anchorage, AK

Brett JR (1995) Energetics. In: Groot C, Margolis L, Clarke WC (eds) Physiological ecology of Pacific salmon. UBC Press, Vancouver, BC, p 3–68

Contreras-Sánchez WM, Schreck CB, Fitzpatrick MS, Pereira CB (1998) Effects of stress on the reproductive performance of rainbow trout (*Oncorhynchus mykiss*). Biol Reprod 58:439–447

Corbel MJ (1975) The immune response in fish: a review. J Fish Biol 7:539–563

Cramer S, Ackerman N, Witty K (2002) Spawning success of hatchery spring Chinook salmon outplanted as adults in the Clearwater River basin, Idaho. Project No. 2001-059000, Report DOE/BP-00006602-1. Bonneville Power Administration, US Department of Energy, Portland, OR

Criscione CD, Watral V, Whipps CM, Blouin MS, Jones SR, Kent ML (2002) Ribosomal DNA sequences indicate isolated populations of *Ichthyophonus hoferi* in geographic sympatry in the north-eastern Pacific Ocean. J Fish Dis 25:575–582

Dykova I, Lom J (1978) Histopathological changes in fish gills infected with myxosporidian parasites of the genus *Henneguya*. J Fish Biol 12:197–202

Eiler JH, Spencer TR, Pella JJ, Masuda MM, Holder RR (2004) Distribution and movement patterns of Chinook salmon returning to the Yukon River basin in 2000–2002. NOAA Tech Memo NMFS-AFSC-148. US Department of Commerce, Springfield, VA

Eiler JH, Spencer TR, Pella JJ, Masuda MM (2006a) Stock composition, run timing, and movement patterns of Chinook salmon returning to the Yukon River basin in 2003. NOAA Tech Memo NMFS-AFSC-163. US Department of Commerce, Springfield, VA

Eiler JH, Spencer TR, Pella JJ, Masuda MM (2006b) Stock composition, run timing, and movement patterns of Chinook salmon returning to the Yukon River basin in 2004. NOAA Tech Memo NMFS-AFSC-165. US Department of Commerce, Springfield, VA

El-Ghany NAA, El-Ashram AMMM (2008) Diagnosis of ichthyophoniasis in *Oreochromis niloticus* in Egypt by polymerase chain reaction (PCR). 8th International Symposium on Tilapia in Aquaculture, p 1307–1328. Available at http://ag.arizona.edu/azaqua/ista/ISTA8/FinalPapers/12/5%20Mycotic%20infections/1%20Ichthyophoniasis%20final.doc

Eriksen MS, Espmark ÅM, Braastad BO, Salte R, Bakken M (2007) Long-term effects of maternal cortisol exposure and mild hyperthermia during embryogeny on survival, growth and morphological anomalies in farmed Atlantic salmon *Salmo salar* offspring. J Fish Biol 70:462–473

Finn JP, Nielson NO (1971) The inflammatory response of rainbow trout. J Fish Biol 3:463–478

Fish FF (1939) Observations on *Henneguya salminicola* Ward, a myxosporidian parastic in Pacific salmon. J Parasitol 25:169–172

Gagliano M, McCormick MS (2009) Hormonally mediated effects shape offspring survival potential in stressful environments. Oecologia 160:657–665

Halos DS, Alexandra H, Hershberger P, Kocan R (2005) *Ichthyophonus* in Puget Sound rockfish from the San Juan Islands Archipelago and Puget Sound, Washington, USA. J Aquat Anim Health 17:222–227

Hamstreet CO (2009) Spring and summer Chinook salmon spawning ground surveys on the Entiat River, 2008. US Fish and Wildlife Service, Leavenworth, WA

Hamstreet CO (2011) Spring and summer Chinook salmon spawning ground surveys on the Entiat River, 2010. US Fish and Wildlife Service, Leavenworth, WA

Jones SRM, Dawe SC (2002) *Ichthyophonus hoferi* Plehn & Mulsow in British Columbia stocks of Pacific herring, *Clupea pallasi* Valenciennes, and its infectivity to chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). J Fish Dis 25:415–421

Kahler E, Burton T, Hamazaki T, Borba BM, Jasper JR, Dehn LA (2007) Assessment of *Ichthyophonus* in Chinook salmon within the Yukon River drainage, 2004. Fishery Data Series No. 07-64. Alaska Department of Fish & Game, Anchorage, AK

Kahler E, Borba BM, Burton T, Dehn LA, Hamazaki T, Jasper JR (2011) Prevalence of *Ichthyophonus* in Chinook salmon entering the Yukon River and Tanana stock spawning grounds, 2004-2006. Fishery Data Series No. 11-11. Alaska Department of Fish & Game, Anchorage, AK

Keefer ML, Peery CA, Heinrich MJ (2008) Temperature-mediated *en route* migration mortality and travel rates of endangered Snake River sockeye salmon. Ecol Freshw Fish 17:136–145

Kent M (2011) Infectious diseases and potential impacts on survival of Fraser River sockeye salmon. Tech Rep 1. Cohen Commission, Vancouver, BC

Kent ML, Benda S, St-Hilaire S, Schreck CB (2013) Sensitivity and specificity of histology for diagnoses of four common pathogens and detection of nontarget pathogens in adult Chinook salmon (*Oncorhynchus tshawytscha*) in fresh water. J Vet Diagn Invest 25:341–351

Kocan RM, Hershberger PK, Winton J (2004a) *Ichthyophoniasis*: an emerging disease of Chinook salmon in the Yukon River. J Aquat Anim Health 16:58–72

Kocan R, Hershberger P, Winton J (2004b) Effects of *Ichthyophonus* on survival and reproductive success of Yukon River Chinook salmon. USFWS Office of Subsistence Management, Fisheries Resource Monitoring Program Final Report (Study No. 01-200). Available at http://alaska.fws.gov/asm/fisreportdetail.cfm?fisrep=22 (accessed 12 March 2013)

Kocan R, LaPatra S, Gregg J, Winton J, Hershberger P (2006) *Ichthyophonus*-induced cardiac damage: a mechanism for reduced swimming stamina in salmonids. J Fish Dis 29:521–527

Kocan R, Hershberger P, Sanders G, Gregg J (2009) Effects of temperature on disease progression and swimming stamina in *Ichthyophonus*-infected rainbow trout *Oncorhynchus mykiss* (Walbaum). J Fish Dis 32:835–843

Kocan R, Dolan H, Hershberger P (2011) Diagnostic methodology is critical for accurately determining the prevalence of *Ichthyophonus* infections in wild fish populations. J Parasitol 97:344–348

Kramer-Schadt S, Holst JC, Skagen D (2010) Analysis of variables associated with the *Ichthyophonus hoferi* epizootics in Norwegian spring spawning herring, 1992–2009. Can J Fish Aquat Sci 67:1862–1873

Loch TP, Scribner K, Tempelman R, Whelan G, Faisal M (2012) Bacterial infections of Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), returning to gamete collecting weirs in Michigan. J Fish Dis 35:39–50

Magnadóttir B (2010) Immunological control of fish diseases. Mar Biotechnol (NY) 12:361–379

Mann RD, Caudill CC, Keefer ML, Peery CA, Schreck CB, Kent ML (2010) Migration behavior and spawning success of spring Chinook salmon in Fall Creek and the North Fork Middle Fork Willamette River: relationship among fate, fish condition, and environmental factors. Tech Rep 2010-7. Cooperative Fish and Wildlife Research Unit, University of Idaho, Moscow, ID

Maule AG, Schrock R, Slater C, Fitzpatrick MS, Schreck CB (1996) Immune and endocrine responses of adult Chinook salmon during freshwater immigration and sexual maturation. Fish Shellfish Immunol 6:221–233

McVicar AH (1999) *Ichthyophonus* and related organisms. In: Woo PTK, Bruno DW (eds) Fish diseases and disorders, Vol 3. Viral, bacterial and fungal infections. CABI Publishing, New York, NY, p 661–687

Mellergaard S, Spanggaard B (1997) An *Ichthyophonus hoferi* epizootic in herring in the North Sea, the Skagerrak, the Kattegat and the Baltic Sea. Dis Aquat Org 28: 191–199

Meyers T, Burton T, Bentz C, Starkey N (2007) Common diseases of wild and cultured fishes in Alaska. ADFG, Fish

Pathology Laboratories, Anchorage, AK

Miller KM, Shulze AD, Ginther N, Li MS, Patterson DA, Farrell AP, Hinch SG (2009) Salmon spawning migration: metabolic shifts and environmental triggers. Comp Biochem Physiol D Genomics Proteomics 4:75–89

Miller KM, Saorong L, Kaukinen KH, Ginther N and others (2011) Genomic signatures predict migration and spawning failure in wild Canadian salmon. Science 331:214–217

Patterson KR (1996) Modeling the impact of disease-induced mortality in an exploited population: the outbreak of the fungal parasite *Ichthyophonus hoferi* in the North Sea herring (*Clupea harengus*). Can J Fish Aquat Sci 53: 2870–2887

Quinn TP, Eggers DM, Clark JH, Rich HB Jr (2007) Density, climate, and processes of prespawning mortality and egg retention in Pacific salmon (*Oncorhynchus* spp.). Can J Fish Aquat Sci 64:574–582

Ramsay JM, Watral V, Schreck CB, Kent ML (2009) *Pseudoloma neurophilia* infections in zebrafish *Danio rerio*: effects of stress on survival, growth, and reproduction. Dis Aquat Org 88:69–84

Schmidt-Posthaus H, Wahli T (2002) First report of *Ichthyophonus hoferi* infection in wild brown trout (*Salmo trutta*) in Switzerland. Bull Eur Assoc Fish Pathol 22:225–228

Schreck CB, Contreas-Sanches W, Fitzpatrick MS (2001) Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture 197:3–24

Sitja-Bobadilla A, Alvarez-Pellitero P (1990) First report of *Ichthyophonus* disease in wild and cultured sea bass *Dicentrarchus labrax* from the Spanish Mediterranean area. Dis Aquat Org 8:145–150

Stephen C, Stitt T, Dawson-Coates J, McCarthy A (2011) Assessment of the potential effects of diseases present in salmonid enhancement facilities on Fraser River sockeye salmon. Tech Rep 1A. Cohen Commission, Vancouver, BC

Tierney KB, Farrell AP (2004) The relationship between fish health, metabolic rate, swimming performance and recovery in return-run sockeye salmon, *Oncorhynchus nerka* (Walbaum). J Fish Dis 27:663–671

Young WP, Blenden M (2011) Chinook salmon (*Oncorhynchus tshawytscha*) spawning ground surveys in the South Fork Salmon River and Big Creek, 1996–2008. www.fws.gov/lsnakecomplan/Reports/NPT/NPT SGS report- SFSR and Big Crk 1996-2008 Final.pdf (accessed 11 July 2013)

Editorial responsibility: Dieter Steinhagen, Hannover, Germany

Submitted: November 28, 2012; Accepted: August 8, 2013
Proofs received from author(s): October 21, 2013