ARTICLE

Synchronous Cycling of Ichthyophoniasis with Chinook Salmon Density Revealed during the Annual Yukon River Spawning Migration

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Abstract

Populations of Chinook salmon Oncorhynchus tshawytscha in the Yukon River declined by more than 57% between 2003 and 2010, probably the result of a combination of anthropogenic and environmental factors. One possible contributor to this decline is Ichthyophonus, a mesomycetozoan parasite that has previously been implicated in significant losses of fish, including Chinook salmon. A multiyear epidemiological study of ichthyophoniasis in the Yukon River revealed that disease prevalence and Chinook salmon population abundance increased and decreased simultaneously (i.e., were concordant) from 1999 to 2010. The two values rose and fell synchronously 91% of the time for female Chinook salmon and 82% of the time for males; however, there was no significant correlation between Ichthyophonus prevalence and population abundance. This synchronicity might be explained by a single factor, such as a prey item that is critical to Chinook salmon survival as well as a source of Ichthyophonus infection. The host–parasite relationship between Ichthyophonus and migrating Chinook salmon from 2004 to 2010 was similar to that reported for the previous 5 years. During 2004–2010, overall disease prevalence was significantly higher among females (21%) than among males (8%), increased linearly with fish length for both males and females, and increased in both sexes as the fish progressed upriver. These regularly occurring features of host–parasite dynamics confirm a stable base of transmission for Ichthyophonus. However, from 2003 to 2010, disease prevalence decreased from 30% to just 8% in males and from 45% to 9% in females, paralleling a similar decline in Chinook salmon abundance during the same period. These findings may help clarify questions regarding the complex host–parasite dynamics that occur in marine species such as herrings Clupea spp., which have less well-defined population structures.

Ichthyophoniasis is a disease of fishes caused by the mesomycetozoan protist Ichthyophonus sp. (Mendoza et al. 2002) and affects both fresh and saltwater species worldwide. (Ichthyophonus sp. is often identified as I. hoferi; however, the original species description [Plehn and Mulsow 1911] was incomplete, and specific criteria for assigning species within this genus are nonexistent [reviewed by McVicar 1999]. Consequently, the organism is hereafter referred to generically as Ichthyophonus.) Although the disease has been studied extensively in Atlantic herring Clupea harengus in the North Atlantic (Scattergood 1948; Sindermann 1970; Kramer-Schadt et al. 2010), Pacific herring C. pallasii in Alaska (Marty et al. 1998, 2010), and haddock Melanogrammus aeglefinus, plaice Pleuronectes platessa, and other saltwater fishes over the past...
century (reviewed by McVicar 1999), there is only speculation as to its impact on morbidity and mortality in wild populations. The major reason for this paucity of morbidity and mortality data is that wild host populations are in a dynamic state, in which fish constantly die and are replaced by younger susceptible individuals. This open-ended situation makes it difficult to quantify how many individuals succumb to ichthyophoniasis (e.g., case-specific mortality) rather than to harvest, predation, starvation, old age, or other diseases. Recruits entering these populations are susceptible to infection, but owing to changing environmental conditions the rate of transmission to these susceptible fish, as well as the parasite dose to which they are exposed, varies from year to year. Consequently, it is difficult to relate specific pathogens to population fluctuations, and this often leads investigators to resort to extrapolation and unsupported assumptions to fill in data voids. An ideal situation to evaluate the impact of ichthyophoniasis on morbidity and mortality would be a population with a finite mortality rate, known causes of mortality, and no susceptible recruits. Salmon populations meet these requirements during their annual spawning migration, when the population is finite, all fish die after spawning, and the same population can be sequentially sampled for diseases during their annual spawning migration.

Sullivan (1989) first reported *Ichthyophonus* from a few Chinook salmon *Oncorhynchus tshawytscha* in the Yukon River; by 2003, the disease had affected 35–45% of returning spawners and was responsible for the loss of approximately 20% of the prespawn population (Kocan et al. 2003, 2004). After these initial studies, disease surveillance was carried out annually from 2004 to 2010 at a reference site near the Rampart Rapids, Alaska, at river kilometer (rkm) 1,170 on the Yukon River. This surveillance program was designed to extend the previous 5-year database and to document changes to *Ichthyophonus* prevalence. Data collection consisted of quantifying clinical disease, which was then used to assess (1) intra- and interannual prevalence, (2) differences in prevalence between sexes, and (3) disease prevalence relative to host size or age. These findings were used to determine whether there was a relationship between annual population density (e.g., run strength) and *Ichthyophonus* prevalence.

**METHODS**

**Fish Sampling**

All Chinook salmon evaluated from 2004 to 2010 were caught from mid-June through mid-August by local fishers using fish wheels and gill nets with 21.6-cm (8.5 in) mesh. After capture, the fish were placed in tanks supplied with flowing creek water at 4–6°C, where they were held until necropsied. At the time of examination, each fish was assigned an accession number and the data from each fish were recorded separately for analysis. Most fish were examined within several hours of capture, but occasional late-day and evening catches were examined the following morning (8–12 h later).

**Tissue Sample Collection**

Each technician responsible for sampling received a full season of training as well as a preseason refresher course each year to standardize the accurate identification of *Ichthyophonus* lesions. Tools and cutting pans were sanitized with 70% alcohol and new sterile disposable blades were used for each fish to avoid cross-contamination. Before tissue sampling and examination, each fish was weighed, and measured; the fish was then opened, the sex was determined, and the viscera was examined for signs (visible lesions) of ichthyophoniasis on the surface of the heart (Figure 1). Fish exhibiting visible cardiac lesions were classified as clinically positive (e.g., diseased), and it was recognized that some low-level as well as subclinical infections may have been missed. All hearts that were putatively positive for *Ichthyophonus* were transported to our base camp, where the project manager re-examined the samples to confirm infection; the manager also processed all samples that were sent to outside laboratories for histological examination or in vitro culture.

**Disease Prevalence**

Disease prevalence was based on visible lesions on the surface of the heart and was determined by the equation:

\[
\text{Prevalence (\%) = 100 \times \left( \frac{\text{number of fish with visible lesions}}{\text{total fish in the sample}} \right)}.
\]

(1)

Annual disease prevalence data (e.g., visible lesions) were then analyzed for (1) intra- and interannual trends, (2) male and
female disease prevalence, and (3) disease prevalence relative to host size. These data were then combined with results from a previous study (Kocan et al. 2004) in order to establish long-term trends and relationships.

Confirmation of Field Diagnoses

Accurate visual identification of *Ichthyophonus* can be problematic if the host is infected with other pathogens that produce similar gross lesions. Conventional methods used to confirm gross diagnoses involve confirmatory tests that verify the identity of the pathogen. To ensure that the observed “lesions” were the result of *Ichthyophonus* infection, three verification methods were used: (1) parasite morphology based on standard histology, (2) histochemistry, and (3) in vitro explant culture of cardiac tissue.

Histology.—Tissues designated for histological evaluation were fixed in 10% neutral buffered formalin, transferred to 70% ethyl alcohol, and then processed for standard single-plane histology with tissue sections cut at 5 µm. The tissues were stained with periodic acid Schiff (PAS) reagent and then microscopically evaluated for the presence of PAS-positive *Ichthyophonus* schizonts (Figure 2). A negative PAS stain would eliminate *Ichthyophonus* as the causative agent; however, because some other pathogens also stain PAS-positive, they had to be distinguished from *Ichthyophonus* on the basis of morphological structures of the organism as well as growth in vitro.

From 2007 to 2010, 50 salmon hearts exhibiting visible lesions were submitted to the Washington State Animal Disease Diagnostic Laboratory in Pullman for histological processing along with 62 hearts that showed no visible lesions. Hearts exhibiting lesions and presumed to be *Ichthyophonus*-positive were microscopically evaluated for the presence of PAS-positive schizonts (arrows; a = spongy muscle; b = dense muscle).

**FIGURE 2.** A histological section of Chinook salmon heart, showing periodic acid Schiff-positive *Ichthyophonus* schizonts (arrows; a = spongy muscle; b = dense muscle).

In vitro explant culture.—As a second means of verifying the accuracy of field diagnoses, hearts with visible lesions (from 36 male and 10 female Chinook salmon) were cultured in Eagle’s minimal essential medium and observed for 21 d for the growth of *Ichthyophonus* germination tubes and an increase in the number of schizonts (McVicar 1982, 1999; Spanggaard et al. 1994; Kocan et al. 2011). The results were then used to confirm the accuracy of the visual field diagnoses. A second subsample of 83 random hearts collected weekly during the 2010 spawning run was also cultured to determine the prevalence of subclinical infections (e.g., infected but no visible lesions).

Disease Prevalence and Population Size

*Ichthyophonus* prevalence data collected at rkm 1,170 (Rampart Rapids) from 1999 through 2010 were compared with Alaska Department of Fish and Game (ADFG) published spawning population abundance for the same years (JTC 2011) to examine possible relationships between the proportion of diseased fish and population abundance. The ADFG classifies returning Chinook salmon as “large” (>65 cm) or “small” (<65 cm); consequently, it was not possible to directly compare male and female disease prevalence with the number of males and females in the run. However, we were able to compare disease prevalence in each sex with total run size.

Synchronous (Concordant) Changes in Population Size and Disease Prevalence

Disease prevalence and fish abundance were compared for annual concordant changes, which are defined as disease prevalence and fish abundance changing in the same direction from one year to the next, with no consideration as to the relative size of the change. Discordance was defined as when the differences in values from one year to the next were of opposite signs.

Data Analysis

Disease prevalence as a percentage of the sampled population (equation 1; ± 95% confidence interval) was determined for each sample group. Comparisons between groups were evaluated with a standard 2 × 2 chi-square distribution with 1 df and significance assigned to $P \leq 0.05$. Linear regression was evaluated by using a coefficient of determination ($r^2$), and synchronicity (concordance) was determined by using a nonparametric test of concordance–discordance.

RESULTS

Numbers of Fish Sampled

During 2004–2010, 5,344 Chinook salmon were examined for visible *Ichthyophonus* lesions at rkm 1,170 on the Yukon River; of these fish, 4,181 (78.3%) were males and 1,163...
FIGURE 3. Relationship between *Ichthyophonus* infection prevalence and Chinook salmon population size in the Yukon River from 1999 to 2010, illustrating a lack of correlation. 

(21.8%) were females. Fish wheels caught 93% (4,967) of the total sample, and gill nets caught 7% (377) of the total.

**Disease Prevalence**

*Disease prevalence versus population size.*—There was no significant correlation ($r^2 = 0.143$) between annual population density and disease prevalence during the 12-year study period (Figure 3).

*Synchronicity.*—Disease prevalence and population abundance rose and fell synchronously in 10 of 11 years (91%) for females (Figure 4) and in 9 of 11 years (82%) for males. Using a null hypothesis of no correlation, we would expect 50:50 concordant: discordant change over time (i.e., null hypothesis $H_0$: $p \leq 0.5$ versus alternative hypothesis $H_a$: $p > 0.5$). Likelihood-ratio tests with a binomial model indicated that it was highly improbable that the concordant changes in prevalence and run strength were random events (two-tailed test: $\chi^2 \geq 8.547$, $P = 0.00346$; one-tailed test of positive correlation: $Z \geq 2.924$, $P = 0.00173$).

*Interannual changes in disease prevalence.*—An increase in disease prevalence from 1999 to 2003 followed by a steady decline through 2010 became apparent when data from this study were combined with data collected at rkm 1,170 during a previous study (Kocan et al. 2003, 2004). Disease prevalence in females decreased from 45% in 2003–2004 to 9% in 2010, and a similar decrease from 30% to 8% occurred in males (Figure 5). The decline in *Ichthyophonus* prevalence corresponded with an annual decrease in host abundance, which declined by more than 57% (from 268,537 to 113,410 fish) between 2003 and 2010 (JTC 2011).

*Intra-annual patterns of disease prevalence.*—Mean disease prevalence for all fish sampled during 2004–2010 was 11.4%, and significantly more females than males were affected ($\chi^2 \geq 12.6$, $P = 0.0001$). Clinical disease increased steadily in both sexes as the spawning migration progressed each year (Figure 6). During the first week of the migration, 6% of males exhibited signs of disease, which increased weekly until disease prevalence peaked at 25% during week 6. Significantly more diseased females were detected throughout the migration, increasing from 11% during week 1 to 54% by week 6. Only nine females were caught during week 7, and these exhibited a disease prevalence of 43%.

*Fish size versus disease.*—There was a positive correlation between clinical disease and total fish length for both sexes (Figure 7). Disease prevalence in males increased from 5% in 50-cm and smaller fish to 15% in 91-cm and larger fish ($r^2 = 0.952$), while disease in females ranged from just under 17% in 61–70-cm fish to 21% in 91-cm and larger fish ($r^2 = 0.833$).
SYNCHRONICITY IN *ICHTHYOPHONUS* AND CHINOOK SALMON ABUNDANCE

**FIGURE 4.** Concordant annual changes in Ichthyophonus prevalence and run size of female Chinook salmon at river kilometer 1,170 of the Yukon River from 1999 to 2010.

**FIGURE 5.** Change in prevalence of clinical ichthyophoniasis in male and female Chinook salmon sampled at Rampart Rapids (river kilometer 1,170) in the Yukon River over a 12-year period (1999–2010), showing a steady decline in disease prevalence from a peak in 2003 to a low in 2010, which paralleled a 57% decline in Chinook salmon population density over the same period. Data from the present study and from a previous study by Kocan et al. (2004) are indicated.
FIGURE 6. Mean (± 95% confidence interval) weekly increase in prevalence of clinical ichthyophoniasis in male and female Chinook salmon sampled at river kilometer 1,170 in the Yukon River during the upriver spawning migration (2004–2010). Sample size (n) is embedded in each column.

FIGURE 7. Comparison of mean (± 95% confidence interval) ichthyophoniasis prevalence in and mean length of male and female Chinook salmon sampled at river kilometer 1,170 in the Yukon River from 2004 to 2010 (sample size n is given in parentheses).
Confirmation of Field Diagnoses

Histology.—Histopathology and histochemistry confirmed 45 (90%) of the 50 Ichthyophonus-positive field diagnoses from 2007 and 2010. Because histology often underestimates the true infection prevalence, this value may be a low estimate of the true positives (Kocan et al. 2011). Based on 62 salmon hearts (clinically negative for Ichthyophonus) that were submitted for histological examination, a subclinical infection prevalence (no visible lesions) of 3.2% was detected in addition to the clinical infections.

In vitro explant culture.—Of 36 male and 10 female hearts identified as clinically positive for Ichthyophonus in the field, 45 (97.8%) were confirmed positive by in vitro culture (Table 1), confirming the accuracy of field diagnoses in this study.

Disease prevalence at rkm 1,170 for the entire 2010 Chinook salmon run was estimated to be 4.6% (n = 989) and was not significantly different (7.2%) from total prevalence (clinical + subclinical) determined by cultured subsamples (n = 83, χ² = 1.71, P = 0.19). Total prevalence values (clinical + subclinical) were not significantly different from total prevalences reported by ADFG for samples taken at the mouth of the Yukon River in 2010 (8.7%; n = 150, P = 0.40) or samples taken upriver from Rampart Rapids (7.0%; n = 199, P = 0.90; JTC 2011), which again confirmed the accuracy of the field diagnoses at rkm 1,170.

Six of the 83 random heart samples cultured positive for Ichthyophonus, and four of these were also clinically positive (Table 2). Subclinical infections detected by explant culture (2.4%) and by histology (3.2%) were not significantly different (n = 128, χ² = 0.65, P = 0.43).

DISCUSSION

Two multiyear epidemiological studies of ichthyophoniasis revealed similar host–parasite relationships relative to sex, size, and migration time in Chinook salmon from the Yukon River. These relationships have held for 12 consecutive years during periods of fluctuating infection prevalence and population size, demonstrating that the basic host–parasite ecology has remained constant. The most intriguing aspect of this host–parasite relationship was the concordance, or direction of annual change, in disease prevalence and population abundance. Even though there was no correlation between population size and disease prevalence, the two events rose and fell synchronously 91% of the time for females and 82% for males, suggesting that they are linked to a common cause.

Cyclical changes in prevalence of ichthyophoniasis and population size have been observed in both Atlantic herring and Pacific herring. For these species, infection prevalences were reported to range from 25% to greater than 50% when population densities were at their peak and then fell to nearly undetectable levels after epizootics, during which the populations declined (Sindermann and Chenoweth 1993; Mellergaard and Spanggaard 1997; Kramer-Schadt et al. 2010). A similar phenomenon was reported in American shad Alosa sapidissima in the Columbia River, Washington, where Ichthyophonus prevalence reached 72% during the period of peak American shad biomass and then fell to less than 50% after an epizootic (Hershberger et al. 2010). The correlation between cyclical population abundance and disease prevalence in herrings and American shad can be explained by case-related mortality, in which infected fish die, leaving a smaller population with a relatively high proportion of uninfected or immune individuals; the population then rebuilds through recruitment of susceptible individuals, and the cycle is reinitiated. Typically, the disease peak precedes peak host abundance and then declines after the loss of infected individuals. This series of events, known as “delayed density dependence,” has been reported in other vertebrates that undergo regular population cycles related to predation (Hanski et al. 1993; Krebs et al. 1995). Parasites can replace predators as drivers of these cycles, as demonstrated in a controlled study that showed that population cycles do result from a single trophic interaction between a parasite and its host (Hudson et al. 1998).

Delayed density dependence does not appear to be responsible for cyclical changes in Chinook salmon in the Yukon River. Rather than the Chinook salmon population declining after a peak in Ichthyophonus prevalence, the two events coincide (e.g., increase or decrease simultaneously), and although the two events appear to be related, the underlying mechanism governing their synchronicity remains unknown. Since all spawning Chinook salmon die every year regardless of their infection history, the prevalence of Ichthyophonus in adults from any given year does not directly influence the prevalence observed in the following year. Therefore, an independent event affecting both

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**TABLE 1.** Correspondence between clinical (visible) Ichthyophonus lesions diagnosed in Chinook salmon in the field and in vitro explants of cardiac tissue from the same individuals.

<table>
<thead>
<tr>
<th>Source</th>
<th>n</th>
<th>Subclinical</th>
<th>Clinical</th>
<th>Total</th>
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<tbody>
<tr>
<td>Field diagnoses</td>
<td>989</td>
<td>ND</td>
<td>45 (4.6)</td>
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</tr>
<tr>
<td>Random sample</td>
<td>83</td>
<td>2 (2.4)</td>
<td>4 (4.8)</td>
<td>6 (7.2)</td>
</tr>
</tbody>
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**TABLE 2.** Comparison of Ichthyophonus disease prevalence (number of fish; percentage in parentheses) between field diagnoses and explant culture of heart tissue from randomly sampled Chinook salmon (χ² = 1.71, P = 0.19).

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<thead>
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Chinook salmon abundance and *Ichthyophonus* prevalence can be postulated; this could be a prey item in the food web that is critical to Chinook salmon survival and acts as a source of *Ichthyophonus* infection.

Each year during the study period (2004–2010), female Chinook salmon exhibited significantly higher disease prevalence than males even though females made up a significantly smaller proportion of the population (9.6% ± 22%), a finding consistent with earlier reports (Kocan et al. 2003, 2004). It is not clear if the low proportion of females is caused by case-specific mortality or some other event (or events). Based on spawning ground and weir counts conducted by various groups during this same 7-year period, the proportion of females in the lower Yukon River was 35 ± 9.6% (mean ± SD), with a range of 16–55% (JTC 2011). The lower proportion of females at rkm 1,170 (middle Yukon River) may be due to different sampling methods or en route mortality of females.

Also consistent with previous findings was a positive correlation between fish size and disease prevalence, which implies that larger (e.g., older) fish are at greater risk of disease related morbidity and mortality. This size or age difference is probably the result of a higher exposure rate among older and larger fish, perhaps resulting from longer feeding time on infected prey or simply consuming more infected prey than smaller fish.

We observed that fish sampled at the end of the run had significantly higher levels of clinical disease than fish at the beginning of the run, even though infection prevalence is constant throughout the run (Kocan et al. 2004); this is consistent with the observations of local fishers and processors, who annually report higher numbers of diseased fish near the end of the run. A plausible explanation for this observation is that decreased swimming performance of infected individuals results in the accumulation of diseased fish near the end of the spawning migration (Tierney and Farrell 2004; Kocan et al. 2006).

Based on 12 years of accumulated epidemiological data, it is evident that many aspects of *ichthyophonia*sis are predictable in Chinook salmon populations returning to the Yukon River. Disease prevalence relative to age, size, sex, and location within the run has been consistent for a 12-year period. There also appears to be a relationship between Chinook salmon spawning population size and *Ichthyophonus* infection prevalence. This relationship manifests as simultaneous or concordant increases and decreases in population size and percentage of infected individuals but does not appear to be a cause-and-effect relationship such as that seen in delayed density dependence. Because this relationship is consistent, it may be possible to use it as a predictive model for changes in either annual run strength or disease prevalence in Chinook salmon.

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