Preliminary Evidence of Sturgeon Density and Other Stressors on Manifestation of White Sturgeon Iridovirus Disease

S. E. LaPatra
J. M. Groff
T. L. Patterson
W. D. Shewmaker
M. Casten
J. Siple
A. K. Hauck

ABSTRACT. Two studies were conducted using 5- to 6-month-old juvenile (mean weight: 3-4 g) white sturgeon, *Acipenser transmontanus*, to examine the effects of density and other stressors on manifestation of disease caused by the white sturgeon iridovirus (WSIV). In the first study, replicate groups of Snake River white sturgeon were stocked at three densities (953, 1,907, and 3,178 fish/m³) in 0.31-m³ fiberglass aquaria with spring water flow through of 30.0 L/minute. No significant (*P* > 0.05) differences were observed among the three groups during the initial 6-week period. However, the high density group cumulative mortality was significantly (*P* <
0.05) greater than the mortality detected in the other groups at 59 days. Pathognomonic signs of WSIV were detected in moribund fish from each group by histological evaluation; however, no virus was isolated. These results suggested that maintaining low sturgeon densities in fish younger than 1 year may be a prudent strategy for minimizing mortality caused by WSIV. In the second study, triplicate or replicate groups of Kootenai River white sturgeon that had been transported for approximately 14 hours at 2-6°C, prior to acclimation at 15°C, were stocked at high and low densities previously tested. No signs of WSIV or abnormal mortality were observed during the initial 2-week period. However, after 36 days the mortality increased to 10-18% and 14-18% in the low and high density groups, respectively, and signs of WSIV disease were detected in moribund fish examined from each treatment by histological evaluation. The presence of the virus was further confirmed by electron microscopy of gill tissue. Although fish density did not appear to affect the occurrence of disease or cumulative mortality, the results suggested that a stressor (e.g., handling, transport, and temperature) in subyearling sturgeon may enhance clinical manifestation of WSIV. [Article copies available from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: getinfo@haworth.com]

INTRODUCTION

The white sturgeon iridovirus (WSIV) was first detected in cultured white sturgeon, *Acipenser transmontanus*, in California in 1988 (Hedrick et al. 1990). The virus has an affinity for epithelial tissue of the integument, gills, oropharynx, and nasal organ. Infected cells become enlarged with subsequent degeneration and necrosis, and infection has been associated with epithelial hyperplasia. Mortality of up to 95% is assumed to be due to anorexia and disruption of normal physiologic processes, including respiratory and osmoregulatory functions. Secondary infections are common, and the disease appears to be most severe in sturgeon younger than 1 year. The original description of WSIV disease occurred in hatchery-raised sturgeon, and the virus was assumed to originate from wild sturgeon adults collected from the Sacramento River and held for use as broodstock. Pathognomonic signs of this disease have been observed in archival histological material from progeny of the first artificially spawned wild stocks, beginning as early as 1983 (Hedrick et al. 1992).

Previous investigations have also reported the presence of the WSIV in cultured white sturgeon from the lower Columbia River in Oregon, the Snake River in southern Idaho, and the Kootenai River in northern Idaho.
LaPatra et al. 1994). In Oregon, WSIV was consistently detected in young sturgeon that were progeny of Columbia River adults and that subsequently had been cultured in river water, but WSIV had not been detected in sturgeon cultured in well water. In Idaho, WSIV has been detected in sturgeon that were progeny of wild Snake River and Kootenai River adults after they had been subjected to stressful conditions of low spring water flows and high fish densities (LaPatra et al. 1994). In the latter observation, mortality subsided when densities were reduced and water flows increased. These observations suggested that WSIV may occur in wild sturgeon and may be present in many Pacific northwest populations, due to the long lifespan of the species, migratory patterns, and continuity of the river systems. Additionally, since the disease appeared size(age)-specific and stress-mediated, fish culture management strategies may potentially be instituted to avoid or minimize epizootics. The purpose of this study was to examine the potential effect of sturgeon density on the manifestation of WSIV disease.

**MATERIALS AND METHODS**

A study was conducted at the College of Southern Idaho (CSI) in Twin Falls, using 1993 brood year Snake River white sturgeon (LaPatra et al. 1994). Replicate groups of 5-month-old sturgeon (mean weight: 3.4 g) were stocked at three densities (953, 1,907, and 3,178 fish/m$^3$) in 0.31-m$^3$ fiberglass aquaria with spring water flow through of 30.0 L/minute. After 59 days, the high and medium density groups were reduced by approximately 50% by transferring fish into additional aquaria. All groups were monitored an additional 4 weeks.

A similar experimental design and protocol were initiated at the Clear Springs Foods (CSF) Research Laboratory using 1993 brood year Kootenai River white sturgeon (Apperson and Anders 1989). Groups of 5.5-month-old sturgeon (mean weight: 3.6 g) obtained from the Kootenai River Sturgeon Hatchery (Bonners Ferry, Idaho) were stocked at the high (11.2 kg/m$^3$) or low (3.2 kg/m$^3$) densities previously tested at CSI. Fish were maintained in aquaria that received ultraviolet-light-disinfected spring water. Triplicate high density groups and replicate low density groups were tested. After 5 months, 1,838 sibling sturgeon remaining at the Kootenai Hatchery with no detectable WSIV or abnormal mortality were transferred to the University of Idaho Aquaculture Research Institute (Moscow, Idaho). Fish were transported at 8°C and acclimated to 15°C upon arrival. These fish were divided into six aquaria on a recirculation
system supplied with chlorinated-dechlorinated well water, cultured at very low densities (0.80-1.12 kg/m³), and monitored for disease.

For all studies, sturgeon mortality was tabulated daily, and specimens were collected for histological examination. Additional moribund animals were collected for viral isolation when mortality increased. Cumulative percent mortality of the replicates was analyzed by analysis of variance on transformed (arcsin/percentage) data (Snedecor and Cochran 1967).

Clinical methods have been described previously (Amos 1985) or are considered general procedures for routine diagnostic examinations of fish. Histological evaluation was routinely performed for diagnosis of WSIV disease. Tissue samples for histological examination were fixed in 10% neutral buffered formalin prior to paraffin embedding, sectioning, and staining (Humason 1979). Histological samples were deparaffinized and processed for transmission electron microscopy when necessary. Specimens were rinsed twice in buffer and then postfixed in 1% aqueous osmium tetroxide, dehydrated through a graded ethanol series, infiltrated, and embedded in epoxy resin. Thin sections (10 to 20 nm) were stained with 4% uranyl citrate and lead acetate prior to examination using a Philips EM4001 transmission electron microscope.

RESULTS AND DISCUSSION

No significant ($P > 0.05$) differences were observed among sturgeon held at different densities at CSI during the initial-6 week period as the densities increased with sturgeon growth (Table 1). However, the cumulative mortality in the high density group was significantly ($P < 0.05$) greater than the mortality detected in the other experimental groups at 59 days. Mean cumulative mortality increased to 7%, 20%, and 57% in the low, medium, and high density groups, respectively, and WSIV infection was diagnosed in all groups by histological examination (Figure 1). The high and medium density groups were subsequently divided into additional aquaria in an attempt to alleviate stressful conditions and minimize mortality. Test groups were monitored for an additional 4 weeks. Cumulative mortality in these latter medium and high density groups increased to 64% and 94%, respectively. Mortality also increased to 26% in the low density groups that had not been divided. Although mortality did not subside in the medium and high density groups after the disease appeared and densities were reduced, sturgeon maintained at the lowest density throughout

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TABLE 1. Mean weight (g) and percent mortality of Snake River white sturgeon after being cultured for 42 days. Values are the means of two replicates. Means were not significantly different ($P > 0.05$).

<table>
<thead>
<tr>
<th>Density designation</th>
<th>Mean weight (g)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (777 fish/m³)</td>
<td>10.3 ± 0.10</td>
<td>2.70 ± 1.33</td>
</tr>
<tr>
<td>Medium (1,871 fish/m³)</td>
<td>9.55 ± 0.15</td>
<td>1.33 ± 0.66</td>
</tr>
<tr>
<td>High (2,966 fish/m³)</td>
<td>8.39 ± 0.21</td>
<td>3.35 ± 0.65</td>
</tr>
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the test period exhibited substantially less mortality. These results and empirical observations suggest that maintaining low sturgeon densities in fish younger than one year may be a prudent strategy for minimizing mortality caused by WSIV.

In the other trial at CSF, sturgeon exhibited no signs of WSIV or abnormal mortality immediately after transfer from the Kootenai Hatchery. However, an increase in mortality of 10-18% and 14-18% occurred in the low and high density groups, respectively, after 36 days, and pathognomonic signs of WSIV disease were detected in moribund fish examined from each treatment by histological evaluation. The presence of the virus was further confirmed by electron microscopy of gill tissue. Sturgeon from the original population maintained at the Kootenai Hatchery showed no evidence of WSIV. Although fish density did not appear to affect the occurrence of WSIV or cumulative mortality, the results suggested that other stressors (e.g., handling and transport) in subyearling sturgeon may also enhance manifestation of WSIV disease.

After 5 months, the remaining sturgeon at Kootenai Hatchery were moved to the University of Idaho Aquaculture Research Institute. No sturgeon had previously been cultured or held at this facility. During the first 10 days mortality among the six groups ranged from 1-7%, and cumulative mortality was 2.7%. However, over the next 60 days as temperatures ranged from 12-19°C, cumulative mortality increased to 58%. Moribund animals exhibited abdominal distention and emaciation and were confirmed to be WSIV-positive by histological examination. In this case, densities again did not appear to be a factor in predisposing fish to a WSIV epizootic, but handling, transport, and temperature may have been involved. Temperature stress may also have been a factor in manifestation of WSIV disease in siblings studied at CSF. These fish were transported
FIGURE 1. Mean number of dead fish detected each day in replicate groups of 5-month-old Snake River white sturgeon (mean weight: 3.4g) stocked at three densities (953, 1,907, and 3,178 fish/m³) in 0.31-m³ fiberglass aquaria with spring water flow through of 30.0 L/minute.
for approximately 14 hours at 2-6°C, prior to acclimation at 14°C over a 6-hour period, and maintained at a constant temperature of 15°C.

As observed in previous studies, subyearling sturgeon appear to be predisposed to WSIV disease by stressors including densities, adverse environmental conditions (e.g., acute temperature fluctuations), and handling. Results from these studies further substantiate the potential for egg-associated and waterborne transmission of WSIV. Although the pathogen may be present, disease may not occur until certain stressors at critical life stages are confronted. We suspect that in each case described in this and previous reports the source of virus infection in cultured juvenile sturgeon was wild sturgeon either caught from the river and used for broodstock or present in the hatchery water supply. However, definitive evidence supporting this hypothesis has yet to be obtained.

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REFERENCES


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