Factors influencing transmission, onset and severity of outbreaks due to white sturgeon iridovirus in a commercial hatchery

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Abstract

Progeny from six different spawns of white sturgeon broodstock were monitored for 20 months in a commercial white sturgeon hatchery for occurrence of outbreaks of white sturgeon iridovirus (WSIV) and white sturgeon herpesvirus-2 (WSHV-2). Five WSIV but no WSHV-2 outbreaks occurred during the study period. Signs of WSIV were restricted to tanks from a single spawn each time (except for one tank during the first outbreak). Temporal–spatial statistical analysis of outbreaks did not indicate that WSIV case tanks were clustered in time and space. Furthermore, WSIV was isolated from progeny of all six spawns participating in the study, even though occurrence of outbreaks and clinical presentation varied greatly among fish from different spawns. Despite failure to identify virus in samples from broodstock, these observations support a hypothesis of vertical transmission of WSIV, with tank-to-tank transmission having a lesser or no role in the spread of the virus. Differences in the onset and severity of WSIV outbreaks in fish from the six participating spawns indicate a possible genetic resistance to the virus and/or a role of stressors. All outbreaks, followed at least one major stressful event that occurred 9–32 days before the appearance of the first disease signs, and simulation modeling showed that the probability of this occurrence being a chance event was 0.14%. We conclude that minimization of stressors (avoidance of pump failures, handling and transportation) of the fish, should be a priority for the hatchery managers. Furthermore, since differences in resistance to WSIV probably exist among spawns, exclusion from reproduction of parents that yielded progeny susceptible to WSIV
White sturgeon iridovirus (WSIV) and white sturgeon herpesvirus-2 (WSHV-2) are the two most important viruses limiting survivability of juvenile white sturgeon. WSIV infections are characterized by anorexia, reduced activity, erratic swimming and increased mortality (up to 95%) (Hedrick et al., 1990, 1992; Watson et al., 1998a). WSHV-2 was first isolated from ovarian fluid of an adult sturgeon and later appeared as a cause of mortality in farmed juvenile white sturgeon (Watson et al., 1995). In contrast to WSIV, WSHV-2-infected fish may not be emaciated, but differentiation of the two infections based on clinical signs is not always reliable (unpublished data). Furthermore, concurrent infections with both viruses can be common in recirculated-water rearing systems (Georgiadis et al., 2000).

Knowledge generated through risk-factor studies is essential to the prevention and control of infectious diseases in fish hatcheries. These studies are critical because infectious diseases in aquaculture are typically multifactorial (Snieszko, 1974; Ahne et al., 1989; Hedrick, 1998). Most published risk-factor studies for diseases in fish hatcheries have investigated bacterial diseases of salmonids: bacterial gill disease in rainbow trout (Bebak et al., 1997) and Aeromonas spp.-related diseases in Atlantic salmon (Jarp et al., 1993) and rainbow trout (Ortega et al., 1996).

In addition, knowledge of transmission patterns of an infectious agent can aid in disease control. Infectious diseases are often characterized by temporal and spatial clustering of cases (Selvin, 1996). Temporal–spatial statistical analysis has been used to elucidate the occurrence and characteristics of the transmission of infectious animal diseases (Paré et al., 1996). While such methods can yield valuable insight into disease transmission in a fish rearing system where water is not recirculated, we are not aware of any studies that used those methods in aquaculture. Our objectives in the present study were to (1) describe outbreaks of WSIV and WSHV-2 in a commercial white sturgeon hatchery, and (2) describe transmission patterns of the two viruses. The overall goal was to provide the knowledge required to formulate prevention and control strategies for the two viral diseases most problematic to white sturgeon aquaculture.
Fig. 1. Hatchery layout and distribution of fish in the tanks during the first outbreak. The WSIV case tanks are indicated by the shaded pattern. The spawn designation (year-spawn number) is given for the fish in each tank. Tank H2 was emptied and filled again during the outbreak with 97-2 fish, which started exhibiting WSIV signs on July 17. Fish in tank K1 (97-1) were asymptomatic for WSIV, but sampled fish were microscopically positive for WSIV.

geon are raised in the hatchery from 1 week (larvae) to 6–13 months (juveniles) of age, when they are transferred to the grow-out room. Fish for this study originated from spawnings conducted at two different sites (a commercial farm and a research institution). We attempted to use single broodstock pairs for all spawnings and to stock each hatchery tank with fish from only one spawning.

2.2. Follow-up and sampling of fish

At spawning, samples of ovarian tissue and semen were collected from broodstock. These samples were processed for inoculation of white sturgeon cell lines for detection of WSIV and WSHV-2 as described by Hedrick et al. (1992) and Watson et al. (1995).

White sturgeon entered the hatchery at the age of 1 week when 3100–5000 fish were stocked in each tank. As fish grew and stocking density increased, fish from each tank were separated into two or more tanks. Fish were monitored daily for signs of viral infection. Dead sturgeon were collected from each tank and counted every 1–2 days. When a viral outbreak was suspected, fish movements in and out of affected tanks were minimized or stopped.

Whenever fish in a tank exhibited signs consistent with WSIV or WSHV-2 infection (reduced feed consumption and one or more of the following signs: increased mortality, slow or erratic swimming, hypochromatic skin lesions), 5–10 clinically affected fish were collected and transported to the Fish Health Laboratory, University of California,
Infrequently, fish samples were collected from hatchery tanks when no disease signs were evident in the hatchery, on occasions just before fish were transported out of the hatchery. Sampled fish were euthanized by being placed in a benzocaine solution (500 ppm) and tissues were excised for histological and virological examination for WSIV and WSHV-2 (Hedrick et al., 1992; Watson et al., 1995; Georgiadis et al., 2000). Sampled tissues included barbell, gills, operculum, mouth, and pieces of skin from the underside of the fish and were pooled for the diagnostic examinations. When fish were too small for the selected tissues to be excised, the entire fish was used, after opening the abdominal cavity with an incision. Tissue sections were stained with H&E and examined microscopically. Virological diagnosis was based on isolation using cell lines derived from white sturgeon skin (WSSK-1) and spleen (WSS-2c1) and observation of virus-specific cytopathic effects (CPE). Examined samples were considered positive for a virus if a positive diagnosis was obtained by either of the two tests for this virus.

When a viral outbreak was suspected, based on evidence of clinical signs, fish were sampled from the affected tanks and from proximal tanks without signs. For a tank to be classified as a potential case we needed to observe at least reduced feeding activity, erratic swimming and lethargy. Anorexia alone was not considered indicative of viral infection, since it can be associated with management (Anita Bunter, personal communication). A tank was considered to have a viral outbreak if the fish it contained presented clinical signs of viral infection and any pooled sample of fish tissues from this tank was positive either by histologic or virologic examination for the respective virus. Often, it was not feasible to collect samples from all tanks that exhibited disease signs. In those instances, fish in tanks that exhibited the same signs at the same time as fish in tanks that were found infected by a specific virus were also considered affected by the same virus. The first day when any of the described signs were evident in a case tank was considered the day of onset of the disease in this tank. All WSIV case tanks in which the onset of clinical signs was up to 17 days apart were considered to belong to the same outbreak. Because this time period is longer than the incubation period observed in experimental infections at similar water temperatures (see below), it was believed to be close to the probable maximum incubation period of disease due to WSIV in natural infections and at the same time provide good resolution of successive outbreaks.

Even though infection-control measures (disinfection of hands and separate siphons and other equipment for each tank) were implemented when infected tanks were detected, there were several occasions when such measures were not followed and transfer of a pathogen among tanks could have occurred. Furthermore, no such precautions were taken when there were no signs of disease. A routine hatchery practice was that when no evidence of disease existed, tanks containing young fish were always cared for first, while during an outbreak, virus-positive or suspected-infected tanks were always cared for last.

2.3. Temporal–spatial clustering analysis

To determine whether tank-to-tank transmission occurred, we tested whether new cases during an outbreak were distributed randomly or were clustered in space and time.
For this purpose we used the Knox test (Knox, 1964a,b). For this test, we needed to specify the maximum incubation period ($T_c$: critical time) and transmission distance ($D$: critical distance) for WSIV in the hatchery.

In experimental studies with WSIV, the time from infection to appearance of clinical signs (incubation period) was dependent on water temperature. At temperatures of 23°C, 19°C, 14°C and 10°C, the incubation period was 7, 10, 20 and 40 days, respectively (Watson et al., 1998b). In another experimental trial conducted at 15°C, the incubation period was 10 days (Hedrick et al., 1992). The water temperature in our study was 19–20°C, however, our fish were probably exposed to lower viral doses than in the experimental challenges. This is expected in field outbreaks, and therefore the incubation period could be longer. For our analysis, we used a maximum incubation period ($T_c$) of 14 days and a $T_c$ of 20 days. Furthermore, we used a $D$ such that transmission could only occur in adjacent tanks or tanks across a corridor, directly but not diagonally opposite. We conducted the Knox tests using the software program CLUSTER (Cluster 3.1, US Department of Health and Human Services, Atlanta, GA).

2.4. Simulation modeling

In an effort to determine whether the proximity of the onset of the five outbreaks to major stressful events (observed 9–32 days prior to disease onset) occurred due to chance, we simulated 50,000 sets of five random dates (corresponding to dates of onset of five hypothetical outbreaks) between the beginning and end of our study period. We then calculated the relative frequency of simulation runs out of the 50,000 in which all five randomly generated dates were between 9 and 32 days from the dates of the stressful events in our study. This relative frequency was considered an estimate of the probability of five random-onset outbreaks happening between 9 and 32 days from a stressful event. This probability can alternatively be considered as the probability that the proximity of stressful events and onset of outbreaks was attributable to chance. We conducted the simulations using @RISK (Palisade, Newfield, NY) and Microsoft Excel (Microsoft, Redmond, WA).

3. Results

During the study period, fish from six spawns (five in 1997, one in 1998) were stocked in the hatchery (Table 1). For three of the six spawns (97-2, 97-5 and 98-1), one male and one female broodstock fish were used (same female but two different males for spawn 97-5). No virus was found in the samples obtained from the respective broodstock fish from the 1997 spawns (the broodstock fish spawned in 1998 were not sampled). Five WSIV but no WSHV-2 outbreaks were detected and WSHV-2 was not recovered from any hatchery fish during our study.

Table 1 has descriptive data about the five WSIV outbreaks and includes dates when important stressful events occurred in the hatchery. Such events were associated with loss of electric power and water pump function and affected all fish that were present in
Table 1

Dates of stressful events and onset of outbreaks and implicated spawns in each outbreak. The number of tanks and number of case tanks refer to the spawn that was implicated in the specific outbreak. Under the heading: “Spawns present”, a plus sign (+) indicates whether fish from a specific spawn were present in the hatchery at each date (if this date referred to the onset of an outbreak, the age of fish is presented instead of a plus sign (+)).

<table>
<thead>
<tr>
<th>Date</th>
<th>Stressful event</th>
<th>Outbreak/implicated spawn</th>
<th>No. of tanks</th>
<th>No. of case tanks</th>
<th>Spawns present (age of fish, in days)</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>97-1</td>
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<tr>
<td>23-Jun-97</td>
<td>+</td>
<td>1st/97-2</td>
<td>17</td>
<td>10 (6/6)</td>
<td>95</td>
</tr>
<tr>
<td>05-Jul-97</td>
<td></td>
<td>2nd/97-4</td>
<td>15</td>
<td>14 (7/8)</td>
<td>156</td>
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<tr>
<td>03-Aug-97</td>
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<td>21-Feb-98</td>
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<td>02-Mar-98</td>
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<td>23-May-98</td>
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<td>11-Jun-98</td>
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</table>

In parenthesis, the number of tanks from which samples tested positive for WSIV using either virology or microscopy over the number of tanks sampled during the outbreak, regardless of whether the specific tank had WSIV signs. Both numerator and denominator refer to fish samples from the spawn implicated in each outbreak.

b Except for one tank containing 97-2 and 97-3 fish and another tank containing 97-1 and 97-3 fish.

c Seventeen tanks contained only 97-2 fish. Out of these, 10 were cases.

d WSIV was also detected in samples from the case tank containing 97-1 and 97-3 fish as well as from the symptomatic tank containing 97-2 and 97-3 fish.

e Fish in the tank from which samples were WSIV-negative were sampled about 1 month before disease onset.

f The tank from the same spawn that gave WSIV positive samples but did not exhibit disease signs was not counted as a case tank but it is included in the numbers in the parenthesis.
the hatchery. They caused fluctuations in water level, and concentration of solids and probably of dissolved oxygen in the tank water. Epidemic curves for the five WSIV outbreaks are shown in Figs. 2 and 3.

3.1. First outbreak

All detected cases of disease were fish from the second 1997 spawn (97-2), except for one tank that contained culled fish from first and third 1997 spawns (97-1 and 97-3). Even though tanks of morbid fish from 97-2 were widely distributed inside the hatchery and interspersed among tanks containing fish from 97-1 and 97-3, the latter fish did not exhibit signs consistent with WSIV infection, except for the one tank mentioned above (Fig. 1).

The first two tanks that presented signs of WSIV infection (one containing 97-2 culs and the other 97-1 and 97-3 culs) on July 5, 1997 were stocked at the highest density of all hatchery tanks at the time. The WSIV was identified in fish from both tanks and the fish were euthanized by the hatchery managers to avoid possible spread of infection. However, on July 18, WSIV signs appeared in other tanks from the same spawn.

Overall, 10 of 17 tanks which contained only 97-2 spawn fish experienced a WSIV episode during the first outbreak. Moreover, the tank with 97-1 and 97-3 fish mentioned above and one tank with 97-2 and 97-3 fish were classified as positive. Fish in five case
tanks were euthanized. In the remaining case tanks, however, signs of viral infection among fish decreased within 1–2 weeks of disease onset. Minimal mortality (zero to a few dead fish in each tank) was observed in all case-tanks.

No fish from other spawns (16 tanks from 97-1, 18 from 97-3, 8 from 97-4) exhibited signs of viral infection. Furthermore, two tanks contained fish from both 97-2 and 97-3 spawns and fish from one of them exhibited signs and were found infected with WSIV. One tank, containing asymptomatic fish from the 97-1 spawn was sampled during the outbreak and microscopic examination of fish tissues revealed a mild subclinical WSIV infection. The Knox test failed to indicate temporal–spatial clustering of cases during this outbreak (for $T_c = 14$, $p = 0.47$; for $T_c = 20$, $p = 0.55$).

### 3.2. Second outbreak

The second outbreak involved only fish from the 97-4 spawn, while fish from other spawns in adjacent tanks did not have signs of viral infection (Fig. 4). All 97-4 fish were eventually transferred to another farm to prevent further spread of infection. Fish in only one of 15 97-4 tanks failed to exhibit WSIV signs before they were transferred to the other farm, but the sample from this tank was positive for WSIV. Fish in this tank were only observed for 4 days after the sampling, when fish were transferred to the other farm. Fish from three tanks were found infected with WSIV at 11, 4 and 4 days,
respectively, before clinical signs appeared. Tanks containing fish that had signs of viral infection and from which WSIV was confirmed had no or very few dead fish before the transfers. The 97-4 fish that were transferred continued showing WSIV signs in the other farm and had 30–70% cumulative mortality. Also, one tank containing 97-1 fish was sampled and was negative for both WSHV-2 and WSIV. Similar to the first outbreak, there was no evidence of temporal–spatial clustering of cases (Knox test: $T_e = 14$ days, $p = 0.38$; $T_e = 20$ days, $p = 0.31$).

Fish from the same spawn (97-4) had been stocked also in another farm where they experienced a WSIV-confirmed outbreak at the age of 50 days. Fish that were moved after stocking within the hatchery in that farm had a more severe outbreak compared with fish that were not moved from their original hatchery tanks, even though they were stocked at higher density.

No disease events were detected in the hatchery between October 17, 1997 and February 15, 1998. During that time, fish from several tanks were sampled, before they were transferred from the hatchery to the initial grow-out room of the farm. These samplings included fish from two 97-1 tanks, five 97-2 tanks (including one that have had signs of WSIV infection during the first outbreak), and five 97-3 tanks. All samples were negative for WSIV and WSHV-2.

### 3.3. Third outbreak

Nine days after an electric power failure at the farm (February 6, 1998), two tanks started exhibiting signs of WSIV infection. Both tanks contained 97-5 spawn fish. The
second of those tanks included fish that had originated from the first tank and had been transferred 2 days before signs appeared. Samples from both tanks were WSIV-positive. The second tank (the one that was transferred) had more severe clinical signs and cumulative mortality of 51%. Fish in those tanks were euthanized on February 27 and 25, respectively. Fish from the same spawn (97-5) that were stocked in another farm also experienced a confirmed WSIV outbreak starting in the first week of February 1998. We did not conduct a Knox test for the third outbreak, because only two tanks were implicated and they both originated from the same tank.

Six of the remaining tanks that contained fish from the 97-5 spawn were sampled between February 21 and March 5, 1998. None contained fish that exhibited disease signs and all samples were negative for WSIV and WSHV-2.

3.4. Fourth outbreak

All fish that exhibited WSIV signs during the fourth outbreak were progeny of the 97-5 spawn. Between February 21 and March 22, six stressful events occurred that affected water supply to the fish tanks, temporarily. On March 25, four tanks that contained 97-5 fish started exhibiting signs of WSIV infection. During the last week of March, the month of April, and the first week of May, 22 of 25 (88%) tanks containing 97-5 fish exhibited signs of WSIV infection. Indeed, two of the three tanks in which fish did not exhibit any signs of WSIV infection were moved on April 7 and 8, and those fish presented WSIV outbreaks in the new tanks where they were transferred 9 and 7 days later, respectively. Moreover, fish in one of the two tanks had been sampled during the transfer and the samples were positive for WSIV. Therefore, there was only one tank with 97-5 fish that was observed until the beginning of June in which fish did not exhibit any clinical signs. Nevertheless, fish in that tank were also found positive for WSIV when sampled on April 7, but WSIV was not detected when sampled again on May 15.

The acute phase of the outbreak (behavioral signs, increased mortality) had ended in all tanks by mid-May, but fish in the tanks were still recovering from the infection (there were still some emaciated fish) during June. Cumulative mortality in this outbreak (from March 25 to May 31, 1998) reached 57% in one tank. The $p$-values for the Knox test were 0.40 and 0.34 for $T_s = 14$ and $T_s = 20$, respectively.

During the fourth outbreak, samples were obtained from fish in two 97-1 tanks (both were positive for WSIV), one 97-2 tank (negative for WSIV), one 97-3 (positive for WSIV) and two tanks with fish from both 97-2 and 97-3 spawns (one positive and one negative for WSIV). None of these tanks exhibited signs of viral infection.

3.5. Fifth outbreak

Yolk sac larvae (98-1 spawn) were stocked in eight hatchery tanks at the time (April 10, 1998) when the fourth outbreak was ongoing. On May 23, the first signs of WSIV infection were detected in fish in one of these tanks and until June 19, fish in all eight tanks exhibited signs of the disease caused by WSIV and increased mortality. During the outbreak (June 10), two new tanks (G5 and 14) were stocked with fish from two of the
eight original hatchery tanks and fish in those tanks started to exhibit WSIV signs on June 19 as well. Finally, all the fish from this spawn (10 tanks) were euthanized. The results of the Knox test for this outbreak using critical times \( T_c \) of 14 and 20 days were nonsignificant (\( p \)-values = 0.32 and 0.49, respectively). For this test only the eight original hatchery tanks were used, because fish in G5 and I4 were transferred into these tanks after the beginning of the outbreak and were stocked away from the other tanks. When we included those tanks in the analysis, the Knox-test \( p \)-values changed minimally.

When we redid the Knox tests for all outbreaks, allowing for potential virus-transmission between tanks that were located diagonally from each other as well as adjacent or directly opposite, all the \( p \)-values increased.

Finally, simulation modeling showed that the probability of obtaining five randomly generated dates, during our study, that occurred within 9–32 days after a stressful event was 0.0014 or 0.14%.

4. Discussion

In the absence of a highly sensitive diagnostic test for WSIV, identification of sources of infection and of individual carrier fish is very difficult in white sturgeon hatcheries. Observation of the patterns of occurrence of disease outbreaks and examination of temporal and spatial clustering of new cases can yield valuable information about the epidemiologic characteristics of a disease. Application of these methods in our study provided evidence that WSIV was vertically transmitted. The presence of WSIV carriers among young fish, the occurrence of stressful events and differences in susceptibility among family groups were important determinants of the onset and severity of outbreaks due to WSIV in the sturgeon hatchery studied. In contrast, tank-to-tank transmission likely played only a minor, if any, role in the occurrence of WSIV outbreaks.

Five WSIV outbreaks occurred in the hatchery, characterized by variable morbidity and mortality. The WSHV-2 was never detected during the study and interviews with farm managers indicated that it had not been isolated from fish in their hatchery for at least 1 year before the beginning of our study. Therefore, all outbreaks were attributed solely to WSIV.

In order to elucidate the mode of transmission of WSIV, we used the Knox test to assess temporal–spatial clustering of cases for four of five outbreaks. The test was not used for the third outbreak because it only included two case tanks. Statistical evidence of clustering would suggest that WSIV was probably transmitted from tank-to-tank (such transmission could happen by water splashed from tank-to-tank or by using WSIV-infected tools). During our study, we tried to prevent these occurrences but this was not always possible, therefore a possibility of transmission existed. The Knox tests did not indicate that WSIV case tanks were clustered simultaneously in time and space. Because the power of the Knox test can sometimes be low (Jacquez, 1996; Paré et al., 1996), nonsignificant statistical findings should not be considered proof of lack of tank-to-tank transmission. However, we believe that we can reasonably conclude that this mode of transmission was of minor importance in the spread of WSIV. If horizontal
tank-to-tank transmission had occurred, we would have expected to observe WSIV disease in tanks adjacent to case tanks that contained fish from different spawns. However, this did not occur except for one tank during the first outbreak (H1, Fig. 1) that contained culls from other than the 97-2 spawn. We therefore concluded that the restriction of WSIV cases to one spawn in each outbreak was attributed to spawn-specific factors (vertical transmission and/or genetic resistance to the disease).

Vertical transmission probably plays an important role in the introduction of WSIV into the facility and in the occurrence of the observed outbreaks. We did not isolate viruses from the broodstock fish that provided offspring for this study, we believe, however, that this was attributable to the low sensitivity of currently available virus isolation methods.

We isolated or detected WSIV in fish from all six spawns, many of which did not experience an outbreak. For the first and third outbreaks, there were no morbid fish in the hatchery for many months before outbreaks began. Furthermore, even if tank-to-tank transmission did occur it is unlikely that the fish affected during the second through fifth outbreaks, which were the youngest fish in the hatchery, were infected from older fish, because the youngest fish were always attended to first. We believe that at least some incoming fish in the facility (at 1 week of age) carried the virus, and were able to transmit it to other fish in the same tank under specific circumstances, which often included the occurrence of a stressful event.

Fish from six tanks that were cases (and/or gave fish to fill tanks that became clinically affected) during the fourth outbreak were WSIV-negative when sampled during or shortly after the third outbreak (as close as 20 days before the onset of the first case of the fourth outbreak). After the fourth outbreak begun, WSIV was found in fish from all sampled tanks containing 97-5 offspring, most of which originated from the six tanks sampled earlier. This probably indicates that some carrier fish in the sampled tanks were harboring virus at an undetectable concentration before the occurrence of the stressful events. We believe that the stressful events triggered the onset of disease in the carrier fish and the spread of the virus to the rest of the tank population.

It is generally difficult to substantiate vertical transmission or a carrier state for a viral infection of fish, especially if available diagnostic tests are not very sensitive. In brook trout, infectious pancreatic necrosis virus (IPNV) can cause a carrier stage and be transmitted vertically (Bullock et al., 1976; Wolf, 1988; Bootland et al., 1991). In white sturgeon, LaPatra et al. (1994) hypothesized that in at least one WSIV outbreak in a white sturgeon hatchery in Idaho, the virus might have been transmitted vertically since infected fish had been hatched and raised in spring water. Hedrick et al. (1992) hypothesized that WSIV was introduced into white sturgeon farms from wild broodstock during the early days of sturgeon farming. They based that claim on histologic detection of WSIV-related lesions in material sampled from the progeny of the first artificially reproduced wild sturgeon.

Although we found WSIV in fish from all six spawns, the occurrence and severity of outbreaks were quite variable, even though fish from all spawns experienced at least one major stressful event. Similar, spawn-related, variability was observed by the hatchery managers during the previous year (1996). One possible explanation would be that there exists genetic resistance to WSIV inherited to the progeny by parent fish.
Fish from the 97-1 and 97-3 spawns did not experience an outbreak, except for one tank (H1) during the first outbreak containing the culled fish from both spawns. The reason why fish in H1 exhibited WSIV disease during the first outbreak is not clear. It is probable that the runt fish contained in H1 were the “weakest” fish of the two spawns. This, combined with the major stressful event that occurred on June 23 and the stress of high stocking density, sorting and transportation 15–21 days before the onset of the outbreak may explain the occurrence of the outbreak, especially if some of those fish might have been WSIV carriers. Furthermore, it is also possible that fish had jumped out of the adjacent case-tank that contained 97-2 fish (H2), when they were incubating or clinically affected with WSIV and mistakenly placed into H1.

Fish from the 97-2 spawn experienced an outbreak of short duration and low mortality while fish from the 97-4 spawn had low mortality before their transfer to another farm but high mortality after the transfer, which included handling and transportation. The 98-1 spawn had a severe outbreak with high mortality (this could be due to the very young age of the affected fish, 46 days). Finally, mortality and clinical presentation were very variable in tanks stocked with fish from the 97-5 spawn during the fourth outbreak.

Matings of one female with two males were used for the 97-5 spawn. There was difference in the clinical presentation and mortality between the offspring of the two fathers during the fourth outbreak, suggesting that the genetic resistance may be partially attributable to the male parent.

Variability in the mortality and severity of disease existed also among offspring of the same parents, and this variability may be associated with the proportion of carrier fish in the individual tank and/or variable intensity of stressors affecting each tank. Stressful conditions have been implicated in the appearance and presentation of WSIV outbreaks in young white sturgeon. Among them, the most important were increased stocking density, water temperature fluctuations, handling and transportation (LaPatra et al., 1994, 1996). In our study, we could always identify a stressful event that preceded the onset of the outbreaks (Table 1). We attempted to quantify the effect of the stressful events on the onset of clinical WSIV disease using a case-crossover design analysis (Maclure, 1991; Marshall and Jackson, 1993). However, we were not able to do that because for most tanks we could not show that fish were at risk for the disease when stressful events happened (which was contingent on whether they were harboring the virus). The affected groups of fish though, in outbreaks 1, 2, 3 and 5 exhibited WSIV disease after experiencing their first major stressful event. We assessed the distribution of outbreak-onset dates relative to stressful events by simulation modeling which indicated that the probability that five randomly picked days of onset were between 9 and 32 days after a stressful event was 0.14%. Because this probability is very low and the observed association is unlikely to have been due to chance, we believe that the stressful events triggered the onset of WSIV outbreaks.

Management factors, such as sorting, transportation, stocking density and physical location of the tank, likely played a role in the timing of the onset and the severity of WSIV outbreaks. In certain occurrences, it was noticed that tanks situated closer to a light or an air-draft source had signs of an outbreak earlier compared to other tanks that were more distant from these sources. Therefore, the additional stresses might have
contributed to the morbidity and mortality, but they can only partially explain the observed variability in clinical presentation.

We did not observe recurrence of viral disease in fish in tanks affected by a previous WSIV outbreak even if, in some cases, they experienced multiple subsequent stressful events. This indicates that fish surviving in tanks where a WSIV outbreak occurred were possibly protected from the disease during subsequent exposures, because of individual or herd immunity.

No WSIV outbreaks were observed in fish younger than 46 days. This is in agreement with hatchery observations in California white sturgeon farms (unpublished observations). Reasons for this finding are unclear. Maternal immunoglobulins have been detected in young white sturgeon from hatching until the 38th day post-hatch (Dr. M. Adkison, University of California, Davis, personal communication) but it is unknown whether these immunoglobulins can protect young fish from WSIV infection or disease.

5. Conclusion

Findings of our study indicate that WSIV is probably vertically transmitted from broodfish to their offspring, while tank-to-tank transmission is not a predominant way of spread of WSIV. If vertical transmission occurs, white sturgeon hatchery managers should manipulate environment and adjust management practices to prevent or alleviate the viral outbreaks. Since, in some cases, infection may be inevitable, minimizing stress factors should be a priority. Furthermore, since there are probably differences in resistance to WSIV among spawns, selection of parent fish and exclusion of iteroporous broodfish that yielded progeny susceptible to WSIV could improve production in white sturgeon hatcheries.

Although our investigation focused on viral outbreaks in white sturgeon, the presented study design and temporal–spatial methods can be applied to infectious diseases in other aquaculture species. Such investigations can provide valuable information on the epidemiology of infectious fish diseases that may not be obtainable otherwise (especially if sensitive diagnostic tests for the infectious agent are unavailable).

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