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Co-infection of *Acipenserid herpesvirus 2* (AciHV-2) and *Streptococcus iniae* in cultured white sturgeon *Acipenser transmontanus*

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ABSTRACT: A mortality event in cultured white sturgeon *Acipenser transmontanus* (Richardson, 1836) sub-adults was investigated. After transfer between farms, high mortality was observed in fish, associated with back arching, abnormal swimming, and ulcerative skin lesions. Necropsy of moribund individuals revealed hemorrhagic ascites and petechial hemorrhages in the coelomic peritoneum and serosa of internal organs. *Acipenserid herpesvirus 2* (AciHV-2) was isolated from external tissue samples, then identified and genotyped by sequencing of the terminase and polymerase genes. In addition, *Streptococcus iniae* was recovered from internal organs of affected fish. Histologic changes were limited to interstitial hematopoietic areas of the kidney and consisted of small foci of necrosis accompanied by fibrin deposition, minimal inflammatory response, and small numbers of bacterial cocci compatible with streptococci. Identity was confirmed by partial sequencing of the 16S rRNA, *rpoB*, and *gyrB* genes. Genetic fingerprinting demonstrated a genetic profile distinct from *S. iniae* isolates recovered from previous outbreaks in wild and cultured fish in North America, South America, and the Caribbean. Although the isolates were resistant to white sturgeon complement in serum killing assays, *in vivo* challenges failed to fulfill Koch's postulates. However, the clinical presentation, coupled with consistent recovery of *S. iniae* and AciHV-2 from moribund fish, suggests viral and bacterial co-infection were the proximate cause of death. To our knowledge, this represents the first report of AciHV-2 and *S. iniae* co-infection in cultured white sturgeon.

KEY WORDS: Acipenserid herpesvirus · *Streptococcus* · Sturgeon

INTRODUCTION

Aquaculture is one of the world's fastest developing food-production sectors. The industry has experienced rapid growth in recent years, as global harvests from wild fisheries have peaked for many species, reaching maximum sustainable yields with scores of wild catch fisheries in decline (Cressey 2009, FAO 2011). Sturgeon aquaculture is particularly important since wild populations have significantly diminished due to overfishing, habitat destruction, and pollution (www.montereybayaquarium.org/animal-guide/fishes/white-sturgeon), with a majority of Acipenseriformes listed as Critically Endan-

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gered (Bronzi & Rosenthal 2014). In the western US, principally in California and Idaho, sturgeon culture is a multi-million dollar industry, generating considerable producer revenues and employment opportunities. The production of high-quality caviar has made this product one of the few aquaculture-generated exports for the US. Ancillary to the value of their eggs and flesh, other value-added commodities have been derived from farmed sturgeon, including medical and health products, cosmetics, and leather (Wei et al. 2011). Overall, North American sturgeon production in 2012 was approximately 1350 metric tons (t), with estimates of US sturgeon production surpassing 2200 t by 2017 and revenues of over US$ 20 000 000 annually.

Infectious diseases pose one of the most significant threats to aquaculture production, resulting in losses of millions of dollars annually (Sommerset et al. 2005, Krkošek 2010). The growth of aquaculture, along with the increased international transport of fish and fish products, has facilitated the emergence and rapid dissemination of several potentially devastating infectious disease agents, including *Streptococcus iniae* (Minchin 2007). In this study we report a co-infection of *Acipenserid herpesvirus 2* (AciHV-2) and *S. iniae* in cultured white sturgeon *Acipenser transmontanus*.

MATERIALS AND METHODS

History and case presentation

For 5 mo, from October 2015 through February 2016, an aquaculture farm in the US Pacific Northwest experienced increased morbidity and mortality in cultured white sturgeon subadults (3 yr old, ~5 kg fish^{-1}). Less than 1 wk prior to the onset of disease, approximately 1000 fish had been transferred from 2 discrete culture tanks at an operation 40 miles (~64 km) away. Water quality parameters on both farms were similar and within acceptable ranges. However, water temperatures at the original farm were consistently lower (average 19°C) than on the farm where mortalities occurred (average 23°C). Clinical signs and gross abnormalities included abnormal swimming behavior, arching of the back, focal to diffuse red skin lesions, abdominal distention, and 30% mortality over a 2 wk period (Fig. 1).

Histological and microbiological analysis

Six white sturgeon from the receiving facility were submitted for diagnostic assessment to the Aquatic Animal Health Laboratory of the University of California – Davis, School of Veterinary Medicine. Fresh tissues were processed for microbiological and histological analysis. Subsamples of brain, spinal cord, liver, spleen, posterior and anterior kidney, stomach, intestine, and gonads were collected and fixed in 10% neutral-buffered formalin. Tissues were processed routinely and stained with hematoxylin and eosin (H&E) for histological examination. Select sections were stained by the Brown and Hopps (Gram) and Giemsa methods.

Samples of posterior kidney and swim bladder lesions were cultured on trypticase soy agar with 5% sheep blood (TSA) and modified Thayer Martin agar plates. Swabs from skin lesions were inoculated onto Shieh medium supplemented with tobramycin. Plates were incubated at 20°C for 15 d. Colonies observed on primary isolation plates were re-streaked for purity, and individual colonies were suspended in sterile 1× phosphate-buffered saline (PBS) with 20% v/v glycerol for cryogenic storage. In addition, subsamples of gill and skin lesions (external tissue pools) and subsamples of posterior kidney and spleen

Fig. 1. Clinical signs and gross lesions in moribund cultured white sturgeon *Acipenser transmontanus* included (A) abdominal distension, arching of body, and (B) focal to diffuse reddened skin lesions

(internal tissue pools) from 6 fish were used to inoculate individual flasks of white sturgeon skin (WSSK-1) cell lines for virus isolation. WSSK-1 cells were incubated at 20°C for 21 d and observed daily for cytopathic effect.

Molecular identification

Bacterial genomic DNA (gDNA) was extracted by suspending a loop of individual bacterial colonies in 500 µl of sterile 1× PBS and following the DNeasy Blood and Tissue Kit (Qiagen) protocol for Gram-positive and Gram-negative bacteria. Extracted DNA was stored at −20°C until further analysis. Initial identification of bacterial isolates was determined by amplification and sequencing of the 16S ribosomal RNA gene using the primers of Dorsch & Stackebrandt (1992). Amplicons were purified with the QiaQuick PCR Cleanup Kit (Qiagen) according to the manufacturer's protocol and submitted to the UC Davis Sequencing Core facility (http://dnaseq.ucdavis.edu/). The obtained sequences were compared with those in GenBank using a BLASTn search of the nonredundant nucleotide (nr/nt) database of the National Center for Biotechnology Information (NCBI). Similarly, DNA samples extracted from virus-infected cell cultures were used in an end-point PCR specific for the DNA polymerase gene of AciHV-2 (Kurobe et al. 2008, T. Kurobe unpubl.).

Genotyping

Repetitive extragenic palindromic PCR (Rep-PCR) fingerprinting of *Streptococcus iniae* isolates from white sturgeon (n = 2) was carried out using the GTG_5 (5′-GTG GTG GTG GTG GTG-3′) primer (Versalovic et al. 1994), following protocols used for typing *S. iniae* (Chou et al. 2014). Genetic profiles were compared to 9 additional *S. iniae* strains representing 3 distinct GTG_5 clusters (Chou et al. 2014) and an *S. iniae* isolate cultured from tilapia in southern California. *Escherichia coli* (ATCC 25922) served as an outlier organism for analysis using the unweighted pair group method with arithmetic mean (UPGMA) based on the Dice similarity coefficient (Quantity One 1-D Analysis Software; Bio-Rad).

Further genetic characterization was accomplished by sequencing the *rpoB* and *gyrB* genes. Primers targeting the genes were developed using the Primer3 utility (Rozen & Skaletsky 2000) in Geneious (Kearse et al. 2012) and synthesized commercially (Eurofins Genomics, Louisville, KY). Primer sequences and thermal cycling parameters are presented in Table 1. Amplicons were purified as above and sequenced commercially (Eurofins Genomics) using the same primers used to generate the amplicons. Ambiguous base calls were manually annotated in Geneious using corresponding chromatograms, and sequence identity was determined by a Blastn search. The *rpoB* and *gyrB* population sets were deposited in GenBank (accession numbers: KY321280–KY321303).

Table 1. Primer sequences and thermal cycling parameters for amplification of fragments of the *rpoB* and *gyrB* genes of *Streptococcus iniae* isolated from cultured white sturgeon *Acipenser transmontanus*

	Primer sequence (5′-3′)
gyrB	
SIgyrB422F	ACA TCG GCA TCG GTC ATK A
SIgyrB1613R	GCG GAG GCG GYT ATA AGG TT
rpoB	
SIrpoB2131F	TTC CGT CGT TCA AAC TCA GG
SIrpoB3220R	TCT CAC CAA AAC GTT GTC CA
Reaction mixture (50 µl)	43 µl of Platinum™ PCR Supermix High Fidelity (Invitrogen), 20 pmol of each primer, 50 ng of gDNA, and nuclease-free water
Cycling parameters	94°C for 3 min, followed by 45 cycles of 94°C for 30 s, 55°C for 30 s, 68°C for 1.5 min, and a final extension of 68°C for 5 min

A portion of the DNA polymerase (1–421 bp) and terminase (1–495 bp) genes from the isolated AciHV-2 (isolate WSHV-15-CA) were sequenced from PCR-amplified DNA fragments by the UC Davis Sequencing Core facility and deposited in the NCBI database (accession numbers: KY440364 and KY 440365). The WSHV-15-CA DNA polymerase sequence was used in a phylogenetic analysis with other sturgeon herpesvirus isolates (Table 2) (Kelley et al. 2005, Kurobe et al. 2008, Kearse et al. 2012). A phylogenetic tree was generated using deduced amino acid sequences of the polymerases (Table 2, see Fig. 7) aligned by MAFFT software ver. 6.814b (algorithm: G-INS-i; scoring matrix: 200PAM/k = 2; gap open penalty: 1.53; offset value: 0.123) (Katoh et al. 2002). Based on the amino acid alignment, gaps were inserted in the corresponding original nucleotide sequences using a custom perl script. The obtained nucleotide alignment was then used for generating a phylogenetic tree using MrBayes program ver. 3.2.1 within Geneious software (ver. 6.1.8) with the following settings: substitution model: Hasegawa-

Table 2. Sturgeon herpesviruses and their DNA polymerase nucleotide sequences used for phylogenetic analysis in this study. A frog herpesvirus, *Ranid herpesvirus 1*, was included for generation of a rooted phylogenetic tree. The region (bp) used for phylogenetic analysis is also shown

Isolate	Source		Accession number	Phylogenetic analysis	Reference
Acipenserid herpesvirus 1					
Reference strain	White sturgeon (farmed)	California, USA	EF685903.1	1–451	Hedrick et al. (1991)
WSHV-03-CA	White sturgeon (farmed)	California, USA	EF685904.1	1–451	Kelley et al. (2005)
Acipenserid herpesvirus 2					
Reference strain	White sturgeon (farmed)	California, USA	AY874416.2	1–421	Watson et al. (1995)
WSHV-15-CA	White sturgeon (farmed)	California, USA	KY440364	1–421	This study
WSHV-99-ID-2	White sturgeon (wild)	Idaho, USA	DQ832173.1	4–424	Kelley et al. (2005)
WSHV-SRWSHV	White sturgeon (wild)	Snake River, USA	FJ815289.2	42,217–42,637	Doszpoly et al. (2011)
SbSHV-2	Not available	Not available	KT183703.1	530–950	Doszpoly & Shchelkunov (unpubl. data)
SSHV-00-CAN	Shortnose sturgeon (farmed)	Canada	EF685906.1	1–421	Kelley et al. (2005)
Ranid herpesvirus 1					
McKinnell	Leopard frog *Lithobates pipiens*	USA	NC_008211	133,289–133,684	Davison et al. (2006)

Kishono-Yano (HKY85); rate variation: propinv; outgroup: RaHV-1; gamma categories: 4; chain length: 10 000 000; heated chains: 4; heated chain temp: 0.5; subsampling freq: 250; burn-in length: 250 000 (Huelsenbeck & Ronquist 2001). The HKY85 model and propinv rate variation were selected by jModelTest ver. 2.1.10 as the best model for the dataset (Guindon & Gascuel 2003, Darriba et al. 2012).

Antimicrobial susceptibility

The minimum inhibitory concentrations (MICs) of a range of antimicrobial agents against the sturgeon *S. iniae* isolates were tested using the Sensititre™ AVIAN Plate format (Trek Diagnostic System). The MICs were determined following the manufacturer's suggested protocol in accordance with standards set forth by the Clinical and Laboratory Standards Institute (2006).

In vitro and *in vivo* challenges

Bactericidal activity of normal and heat-inactivated white sturgeon serum was tested against the sturgeon *S. iniae* isolates. Blood was collected from 5 healthy subadult fish maintained at the UC Davis Center for Aquatic Biology and Aquaculture by caudal venipuncture using 3 ml red top Vacutainer tubes (BD Vacutainer Systems) with no anticoagulant. Prior to collection, fish were anesthetized in 150 mg ml^{-1} MS-222 (Western Chemical). Blood was allowed to clot for 4 h at 4°C and centrifuged at 3000 × *g* (10 min) to separate serum. A sub-sample of the sera was heated in a water bath at 55°C for 30 min to inactivate complement. *S. iniae* isolates, cultured as previously described, were adjusted to a concentration of 1×10^7 colony-forming units (CFU) ml^{-1} in PBS, and equal volumes of the bacterial suspensions and either normal or heat-inactivated serum were combined and incubated at room temperature. At 0, 1, and 2 h, sub-samples were collected, serially diluted in PBS, and spot plated on TSA plates. Viable CFUs were estimated by the drop plate method on TSA using 50 µl drops of each 10-fold dilution (Quinn et al. 2011).

To fulfill Koch's postulates, experimental challenges were performed by intracoelomic injection (IC) with *S. iniae* from the diseased sturgeon following approval by the UC Davis Institutional Animal Care and Use Committee. Briefly, isolates were revived from cryogenic storage by streaking on TSA. Harvested cells were suspended in PBS to approximate a 0.5 McFarland standard and CFU enumerated using the drop plate method described above. Naïve, healthy white sturgeon (average weight 150 g) were obtained from a source with no history of infection. Fish were maintained at a density of 10 $tank^{-1}$ in separate challenge and control tanks receiving 18 ± 1°C, aerated, fresh water at a flow rate 1.5 l min^{-1}. Prior to challenge, all fish were anesthetized with MS-222 (150 mg l^{-1}). Challenged fish were each injected with 0.1 ml of *S. iniae* suspension containing ~10^7 CFU. Control fish received the same volume of sterile PBS. Following challenge, fish were returned to their respective tanks, and mortality was recorded every 12 h for 30 d.

RESULTS

Gross and microscopic findings in naturally infected white sturgeon

Necropsy of moribund, naturally affected fish revealed ecchymoses on the ventral surface of the head, operculum, and fin bases, with marked swelling of the pectoral fins of some fish, as well as erosions and ulcers of varying severity in the skin and fins (Fig. 2). Internal changes, including hemorrhagic ascites and petechiation of the coelomic wall and visceral serosa, were consistent with septicemia (Fig. 3A,B). Extensive hemorrhagic ulcers surrounded the internal opening of the swim bladder pneumatic duct (Fig. 3C,D).

Histopathologic changes in naturally affected fish were limited to the posterior kidney, consisting primarily of scattered foci of necrosis involving individual and small groups of cells within interstitial hematopoietic areas. Occasional larger foci of necrosis, often in periarterial locations, included edema and deposition of pale eosinophilic, fibrin-like material. Bacteria could not be reliably distinguished from pyknotic debris using routine H&E stains (Fig. 4A,B). However, with Giemsa and Gram stains, small numbers of Gram-positive cocci in pairs and short chains were widely scattered throughout interstitial areas. Bacteria were primarily non-cell associated and elicited little or no inflammatory response (Fig. 4C,D). Lesions were not observed in the brains, spinal cords, livers, spleens, hearts, anterior kidneys, or gonads of the fish examined.

Microbiological analysis

Abundant pin-point colonies of catalase-negative, β-hemolytic, Gram-positive, chain-forming bacteria, indicative of *Streptococcus* spp., were consistently isolated from swim bladders (3/6) and posterior kidneys (5/6) 3 to 5 d post-inoculation on TSA agar. The *Streptococcus* spp.-like organism failed to grow on Shieh media. Other organisms were occasionally isolated on TSA and Shieh agar.

Clusters of round cells, consistent with AciHV-2-induced CPE, were observed in WSSK-1 cell lines inoculated with pooled gill and skin samples from 5/6 fish after 3 to 9 d incubation. End-point PCR of the AciHV-2 polymerase gene confirmed the preliminary diagno-

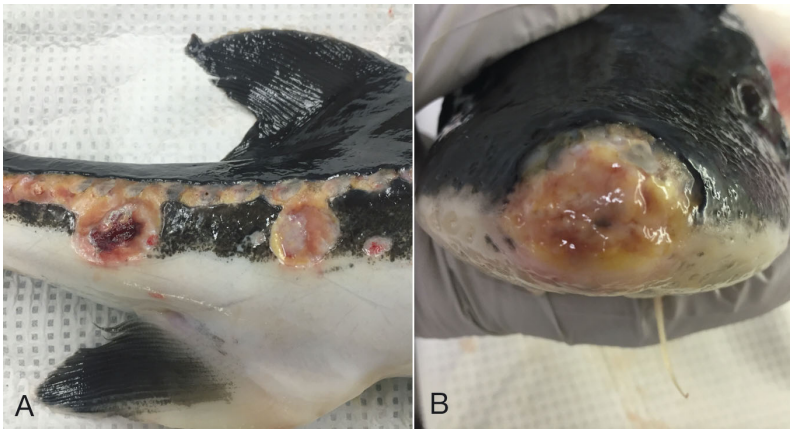

Fig. 2. Multiple (A) ulcerative skin and fin lesions and (B) proliferative lesions, primarily in the snout area, were observed in affected white sturgeon *Acipenser transmontanus* sub-adults during necropsy

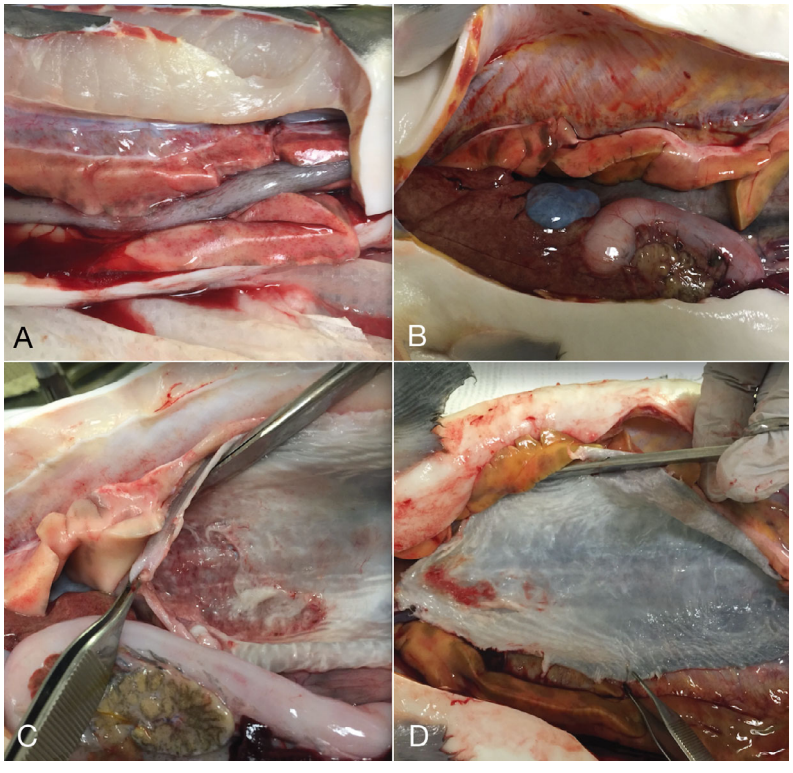

Fig. 3. Internal gross pathologic findings in white sturgeon *Acipenser transmontanus* included (A,B) bloody ascites, petechiae, and ecchymoses in multiple organs, consistent with septicemia, and (C,D) extensive ulcerative hemorrhages surrounding the pneumatic duct in swim bladders

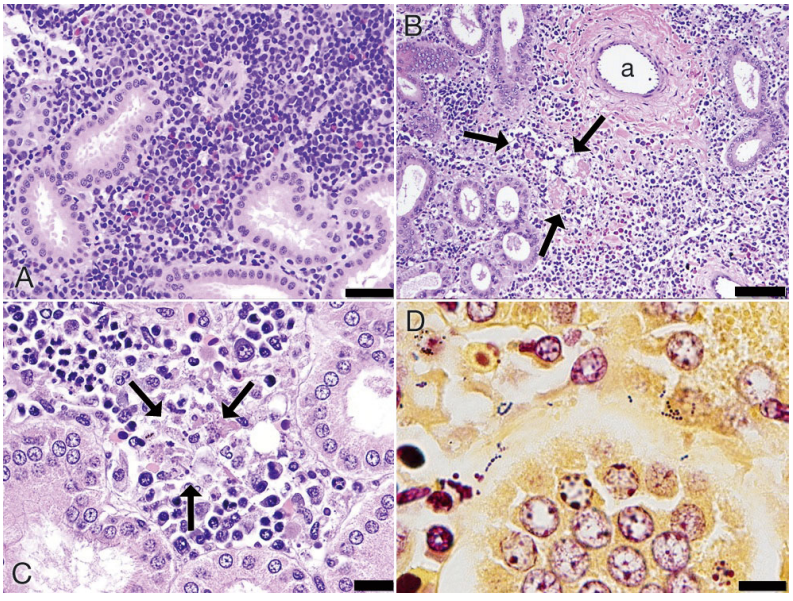

Fig. 4. Histologic sections of white sturgeon *Acipenser transmontanus* kidney. (A) Normal tissue from a healthy fish with renal tubules embedded within abundant, highly cellular interstitial hematopoietic tissue (H&E). Scale bar = 50 µm. (B) *Streptococcus iniae*-infected kidney collected from a moribund fish. Hematopoietic tissues surrounding an artery (a) are hypocellular and edematous. The lumen of an adjacent vein is partially occluded by necrotic cellular debris and pale eosinophilic fibrin (arrows) (H&E). Scale bar = 100 µm. (C) Focal necrosis (arrows) within hematopoietic tissue (H&E). Scale bar = 20 µm. (D) Small numbers of Gram-positive cocci in short chains, compatible with *S. iniae*, free within interstitial tissue (Brown & Hopps). Scale bar = 10 µm

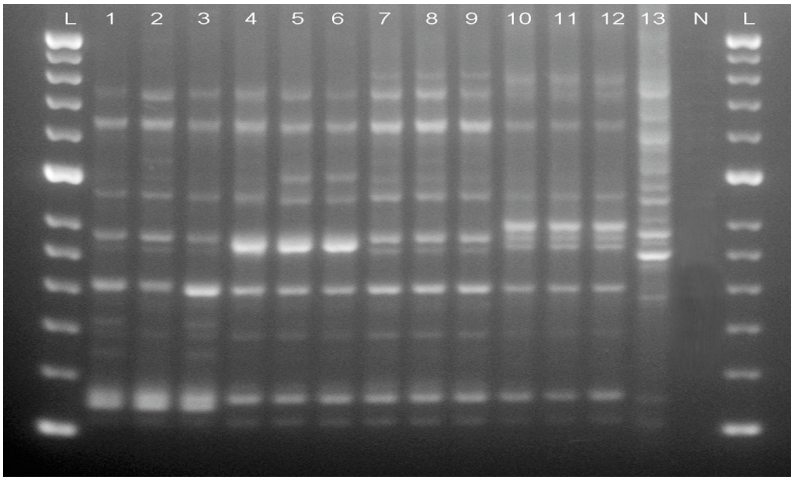

Fig. 5. Genotyping of *Streptococcus iniae* isolates recovered from cultured white sturgeon *Acipenser transmontanus* and representative genogroups from a previous study (Chou et al. 2014). Repetitive extragenic palindromic PCR amplification of DNA from *S. iniae* (Lanes 1–12), and *Escherichia coli* (Lane 13) isolates. Genetic profiles were generated using GTG_5 primers. Lane designations are as follows: L = Hyperladder™ 50 bp; 1 = WS-6B; 2 = WS-6H; 3 = F15-4-3; 4 = ARK PB 03-62B; 5 = LSU 10-070; 6 = LSU 01-105; 7 = LSU 94-034; 8 = LSU 94-036; 9 = LSU 96-325; 10 = B8; 11 = B7; 12 = K08-409H; 13 = *E. coli*; and N = negative control

sis (T. Kurobe unpubl.). Amplification and partial sequencing of 16S rRNA identified the isolates as *S. iniae*, sharing 99% identity over 957 bp with other *S. iniae* sequences available in GenBank. The MICs of oxytetracycline, florfenicol, and trimethoprim/sulfamethoxazole were ≤0.25, ≤1, and ≤0.5/9.5 µg ml^{-1}, respectively. Other sporadically recovered organisms were identified from 16S rRNA sequences as *Flavobacterium columnare*, *Shewanella putrefaciens*, *Aeromonas hydrophila*, and *Chryseobacterium* spp. after amplification and partial sequencing of the gene. Additionally, a suspected oomycete, morphologically consistent with *Saprolegnia* spp., was recovered from 1 skin lesion.

Bacterial and viral genotyping

Rep-PCR-generated profiles consisted of 7 to 11 bands of varying intensity ranging in size from 300 to 2000 bp (Fig. 5). The white sturgeon isolates formed a single distinct cluster, which included the *S. iniae* tilapia isolate from southern California. A dendrogram derived from UPGMA cluster analysis using the Dice similarity coefficient identified 4 distinct clusters (90% similarity cutoff), with the California isolates forming a discrete phylogroup separate from other representative *S. iniae* GTG_5 clusters from a previous study (Fig. 6) (Chou et al. 2014). All isolates demonstrated a high sequence similarity to those of *S. iniae* strain YSFST01-182 (GenBank CP010783) (Rajoo et al. 2015) at both *rpoB* (973 bp; 100% similarity) and *gyrB* (1064 bp; 99.8–100%), confirming that the genetic variability identified by rep-PCR was intraspecific.

The DNA polymerase and terminase genes of the AciHV-2 isolate WSHV-15-CA were identical to those of the AciHV-2 reference strain, WSHV-99-ID-2, as well as WSHV-SRWSHV (Table 3). The phylogram of DNA polymerase genes placed iso-

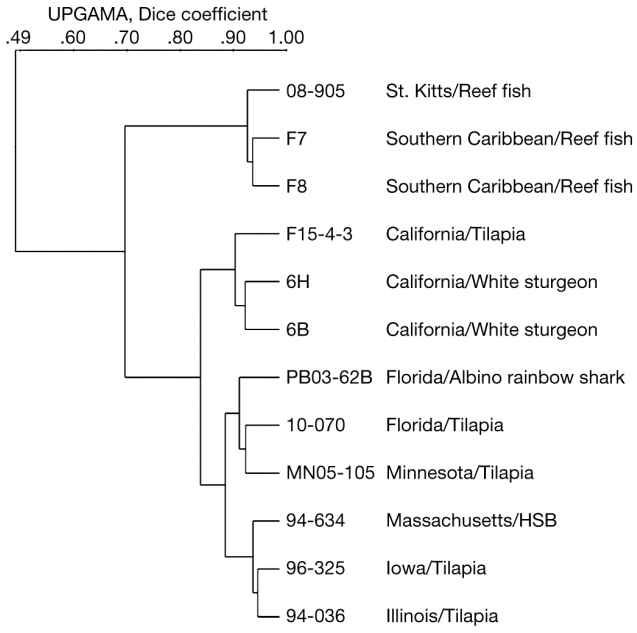

Fig. 6. Dendrogram derived from UPGMA cluster analysis using the Dice coefficient matrices generated from repetitive extragenic palindromic PCR amplification of *Streptococcus iniae* DNA (Fig. 5) and rooted at *E. coli*. Isolates from white sturgeon *Acipenser transmontanus* cultured in California form a discrete phylogroup that includes an *S. iniae* isolate from tilapia in California and is separate from other *S. iniae* phylogroups from previous studies (Chou et al. 2014)

late WSHV-15-CA in the clade of AciHV 2 and other type 2 herpesviruses, including the closely related isolates, SbSHV-2 and SSHV-99-CAN (Fig. 7). The relationships among the sturgeon herpesviruses were strongly supported by high posterior probabilities (>0.80) (Fig. 7).

In vitro and *in vivo* challenges

S. iniae isolates demonstrated complete resistance to serum killing by both heat-inactivated and normal white sturgeon serum, as the number of bacteria re-isolated from the wells was similar to the number inoculated. Koch's postulates were not fulfilled utilizing the *in vivo* challenge described. No mortalities occurred during the 30 d challenge, and no bacteria were recovered from the posterior kidney of fish surviving to the end of the experiment.

DISCUSSION

An increasing number of viral and bacterial diseases are being reported in both wild and cultured

Table 3. Pairwise sequence similarity comparisons of DNA polymerase nucleotide sequences for type 2 sturgeon herpesvirus isolates. The numbers in the table indicate percentage of sequence similarities at the nucleotide level

		2	3	4	5	6
1	WSHV2-15-CA	100	100	100	94.8	88.4
2	AciHV2, Reference strain		100	100	94.3	88.4
3	WSHV-99-ID-2			100	94.2	88.4
4	WSHV-SRWSHV				92.3	88.4
5	SbSHV-2					88.0
6	SSHV-99-CAN					

sturgeon. Herpesviral (AciHV-1 and AciHV-2), iridoviral, and iridoviral-like infections are among the most commonly reported causes of mortalities, including emerging pathogens such as ranaviruses and the nucleo-cytoplasmic large DNA viruses (Hedrick et al. 1990, 1991, Watson et al. 1995, Georgiadis et al. 2000, La Patra et al. 2014, Waltzek et al. 2014, Ciulli et al. 2016). Reports of bacterial diseases in sturgeon are relatively few, with most involving opportunistic infections by motile aeromonad species in association with environmental stressors (Ciulli et al. 2016). Diseases attributed to members of the *Streptococcaceae* include *Lactococcus lactis* subsp. *lactis* in cultured *Huso huso* × *Acipenser ruthenus* in Taiwan (Chen et al. 2012), as well as *Streptococcus dysgalactiae* in cultured Chinese Amur sturgeon *A. schrenckii* and *S. iniae* in hybrid *H. dauricus* × *A. schrenckii* and Siberian sturgeon *A. baerii* (Yang & Li 2009, Wang et al. 2014, Deng et al. 2017). *S. iniae* has not been pre-

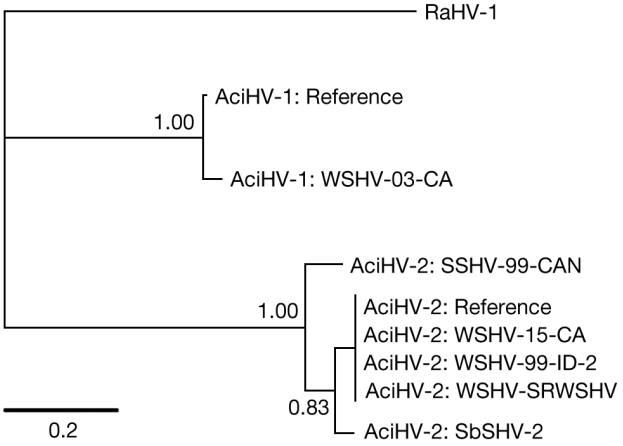

Fig. 7. Phylogenetic tree of sturgeon herpesviruses. The tree was generated with DNA polymerase nucleotide sequences of sturgeon herpesviruses by MrBayes program ver. 3.2.1 (Table 2). Posterior probabilities are shown at each node. The scale bar indicates estimated substitutions per site. Ranid herpesvirus (RaHV)-1 was used as an outgroup

viously reported from any sturgeon species in North America.

Consistent isolation of *S. iniae* and AciHV-2, coupled with gross and microscopic findings, suggest co-infection as the most likely cause of death in these white sturgeon. Although the farm origin of the bacteria is unknown, the epizootic was likely precipitated by transport stress and potentially exacerbated by temperature differentials between the 2 culture facilities. Sturgeon, similar to other fish species, likely become immunosuppressed in association with suboptimal environmental conditions and poor husbandry, increasing their susceptibility to infection (Wedemeyer 1996). For example, chlorine exposure is suspected to have precipitated herpesviral and iridoviral co-infection in cultured shortnose sturgeon *A. brevirostrum* (L.) (La Patra et al. 2014).

S. iniae is a zoonotic Gram-positive bacterial pathogen of cultured and wild fish (Agnew & Barnes 2007). Poor husbandry and environmental stressors, such as high stocking densities, elevated ammonia, low dissolved oxygen, and parasitism, as well as suboptimal pH, salinity, and water temperatures, are known risk factors for fish streptococcosis (Xu et al. 2009, Zamri-Saad et al. 2014). AciHV-2 causes chronic, recurrent hyperplastic and erosive dermatitis in farmed white sturgeon juveniles and subadults. Not typically associated with high mortality, cutaneous lesions may be colonized by opportunistic bacteria and fungi. Disease recrudescence may occur, and like *S. iniae* outbreaks, may also be precipitated by farming practices and other stressors (Watson et al. 1995).

While *S. iniae* infections are more common in warmwater fish species, it is a significant pathogen of rainbow trout *Oncorhynchus mykiss* cultured at 16°C (Lahav et al. 2004, Eyngor et al. 2008, Erfanmanesh et al. 2012). Mozambique tilapia *Oreochromis mossambicus* exposed to suboptimal water temperatures exhibited decreased leucocyte counts, respiratory burst, phagocytic activity, and phagocytic index, leading to immunosuppression and increased mortality following challenge with *S. iniae* (Ndong et al. 2007). While similar effects are likely to occur in white sturgeon, information is limited regarding the effects of temperature on immune responsiveness and disease susceptibility. In natural habitats, they encounter temperatures of 8 to 12°C as they migrate from bays to upper river systems to spawn (Conte et al. 1988). However, growth, sexual maturation, and times to caviar production can be accelerated in culture operations by increasing temperatures to 20–25°C (Cech et al. 1984).

The white sturgeon *S. iniae* isolates shared high degrees of similarity at multiple gene loci (16S, *rpoB*, and *gyrB*) to those from previous studies, indicating they are conspecific. However, genetic fingerprinting identified a discrete genotype that was similar to *S. iniae* recovered from tilapia in southern California, yet distinct from isolates associated with epizootics in North America, South America, and the Caribbean. This suggests that a unique genetic variant may be associated with disease outbreaks in the Pacific Northwest. While the white sturgeon *S. iniae* isolates were susceptible to antimicrobial agents approved for use in US aquaculture in other fish species, *in vitro* susceptibilities and the potential for clinical efficacy are difficult to assess, as criteria for interpreting MIC results for *S. iniae* have not been established.

The DNA polymerase and terminase gene sequences obtained from the AciHV-2 isolated WSHV-15-CA were identical to those of AciHV-2 isolated previously in California (reference strain) and Idaho (WSHV-99-ID-2) (Tables 2 & 3). WSHV-99-ID-2 was isolated from wild sturgeon from the Columbia and Snake River drainages in Idaho. As described in earlier publications, wild adult white sturgeon were introduced from these systems to fish hatcheries in northern California and represent a possible source of the AciHV-2 found in this study (Watson et al. 1995, Kurobe et al. 2008).

The development of a reliable challenge model to reproduce the disease is needed to gain a more thorough understanding of the pathogenesis of *S. iniae* infection in cultured white sturgeon. While Koch's postulates were not fulfilled, challenge parameters did not simulate typical farm conditions. The fish used were healthy, younger, and acclimatized for several weeks in tanks at a lower temperature and stocking density than at the aquaculture facility. The fish were also not concurrently infected with AciHV-2. It is possible that the *S. iniae* isolate is weakly pathogenic and only capable of causing opportunistic infections in white sturgeon. It produced atypically mild lesions in naturally infected fish, and nervous system involvement was not observed in the specimens examined. In contrast, *S. iniae* isolates from Siberian sturgeon in China produced typical lesions and lethal dose (LD_{50}) values of (5.1–6.4) × 10^5 CFU $fish^{-1}$ following intraperitoneal injection at 24–26°C (Deng et al. 2017). In natural infections, AciHV-2 may have facilitated entry of the bacterium through damaged skin or caused antagonism of the host immune response (Rinaldo 1990, Alibek et al. 2014). Similarly, Ciulli et al. (2016) reported high mortality in cultured Russian *A. gueldenstaedtii* and Siberian

sturgeons concurrently infected with an epitheliotropic nucleo-cytoplasmatic large DNA virus, *Aeromonas* spp., and *Acinetobacter* spp. Future research investigating interactions between the 2 agents, including routes of infection, transmission, and pathogenicity, are warranted to support continued expansion of sturgeon aquaculture in North America and protect wild fish stocks.

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