ELSEVIER

Aquaculture 126 (1994) 201–210

Aquaculture

Occurrence of white sturgeon iridovirus infections among cultured white sturgeon in the Pacific Northwest

S.E. LaPatra[a,*], J.M. Groff[b], G.R. Jones[a], B. Munn[c], T.L. Patterson[d], R.A. Holt[e], A.K. Hauck[f], R.P. Hedrick[b]

[a]*Clear Springs Foods, Inc., Research Division, P.O. Box 712, Buhl, ID 83316, USA*
[b]*Department of Medicine, School of Veterinary Medicine, University of California, Davis, CA, USA*
[c]*Department of Pathology, School of Medicine, University of California, Davis, CA, USA*
[d]*College of Southern Idaho Aquaculture Program, Twin Falls, ID, USA*
[e]*Oregon Department of Fish and Wildlife, Department of Microbiology, Oregon State University, Corvallis, OR, USA*
[f]*Idaho Department of Fish and Game, Eagle Fish Health Laboratory, Eagle, ID, USA*

Accepted 4 May 1994

Abstract

The white sturgeon iridovirus (WSIV) was detected in cultured white sturgeon *Acipenser transmontanus* from the lower Columbia River in Oregon, the Snake River in southern Idaho and the Kootenai River in northern Idaho, USA. In Oregon, WSIV was consistently detected in young sturgeon that were progeny from Columbia River adults and cultured in river water but not detected in sturgeon cultured in well water. In Idaho, WSIV was detected in sturgeon that were progeny from wild Snake River and Kootenai River adults after being subjected to stressful conditions of low spring water flows and high fish densities. When densities were reduced and water flows increased, mortality subsided. These observations suggest that WSIV may occur in wild sturgeon and that the virus may be present in many Northwest populations due to the long life span of the species, migratory patterns, and continuity of the river systems. Additionally, since the disease appears size(age)-specific and stress-mediated, fish culture management strategies could be used to avoid or minimize epizootics. These include iodophor disinfection of eggs, sustaining low fish densities and loadings, maintaining virus-free water supplies, minimizing adverse environmental conditions, and reducing the handling of sturgeon younger than 1 year.

Keywords: White sturgeon; Iridovirus; WSIV

* Corresponding author.

SSDI 0044-8486(94)00117-7

1. Introduction

In the United States, development of hatchery technologies for the culture of white sturgeon (*Acipenser transmontanus*) has led to a small but growing commercial industry in California, Idaho, Oregon, and Washington. The largest portion of the industry is located in northern California where sturgeon are produced as a food fish and secondarily as an ornamental fish used in the aquarium trade. Current production in California is estimated at 1 million pounds per year of fish for the food market with an estimated value of $4 million (Serge Doroshov, University of California, Davis, unpublished observation). State resource agencies are also interested in hatchery-produced white sturgeon for sport fishery enhancement, to mitigate for broodstock taken from wild populations, and to restore threatened and endangered stocks.

Among potential problems hindering the continued development of sturgeon aquaculture are viral diseases that cause mortality among fish at early life stages (Hedrick et al., 1985; Hedrick et al., 1990, 1991a. One such virus, the white sturgeon iridovirus (WSIV), was first detected in cultured white sturgeon in California in 1988 (Hedrick et al., 1990). The virus has an affinity for the epithelium of the integument, gills, oropharynx, and nasal organ. Infected cells become enlarged with subsequent degeneration and necrosis. Mortality due to this disease can be high and secondary bacterial and protozoal infections are common. The disease is most severe in young sturgeon and generally not observed in fish older than yearlings. Diagnosis of WSIV is commonly done histologically by observing amphophilic to basophilic enlarged epithelial cells in affected tissues. Confirmation is by ultrastructural analysis (Hedrick et al., 1992). Although sometimes difficult, the virus has been isolated using a sturgeon spleen cell line (WSS-2; Hedrick et al., 1991b) and serum neutralization tests have been used for the confirmation of WSIV from infected cell cultures.

The virus that infected hatchery-reared sturgeon in California is believed to originate from wild sturgeon adults collected from the Sacramento River and held for broodstock. This is supported by observation of infections in archival histological material from some of the first artificially-spawned progeny from these wild stocks beginning as early as 1983 (Hedrick et al., 1992). The purpose of this report is: (1) to describe the WSIV epizootics that occurred in cultured white sturgeon from the lower Columbia River in Oregon, the Snake River in southern Idaho and the Kootenai River in northern Idaho, USA; (2) to assimilate the epizootiological information and suggest sources of WSIV; and (3) to propose fish culture management strategies to minimize disease due to WSIV.

2. Material and methods

Epizootics

Lower Columbia River, Oregon. During August and September, 1990, several samples of juvenile white sturgeon from a private hatchery located about 5 miles downriver from Bonneville Dam at Dodson, OR, were submitted for clinical examination (Fig. 1). The water supply to the hatchery at Dodson was pumped Columbia River water. The sturgeon juveniles were progeny from four lower Columbia River white sturgeon females, one

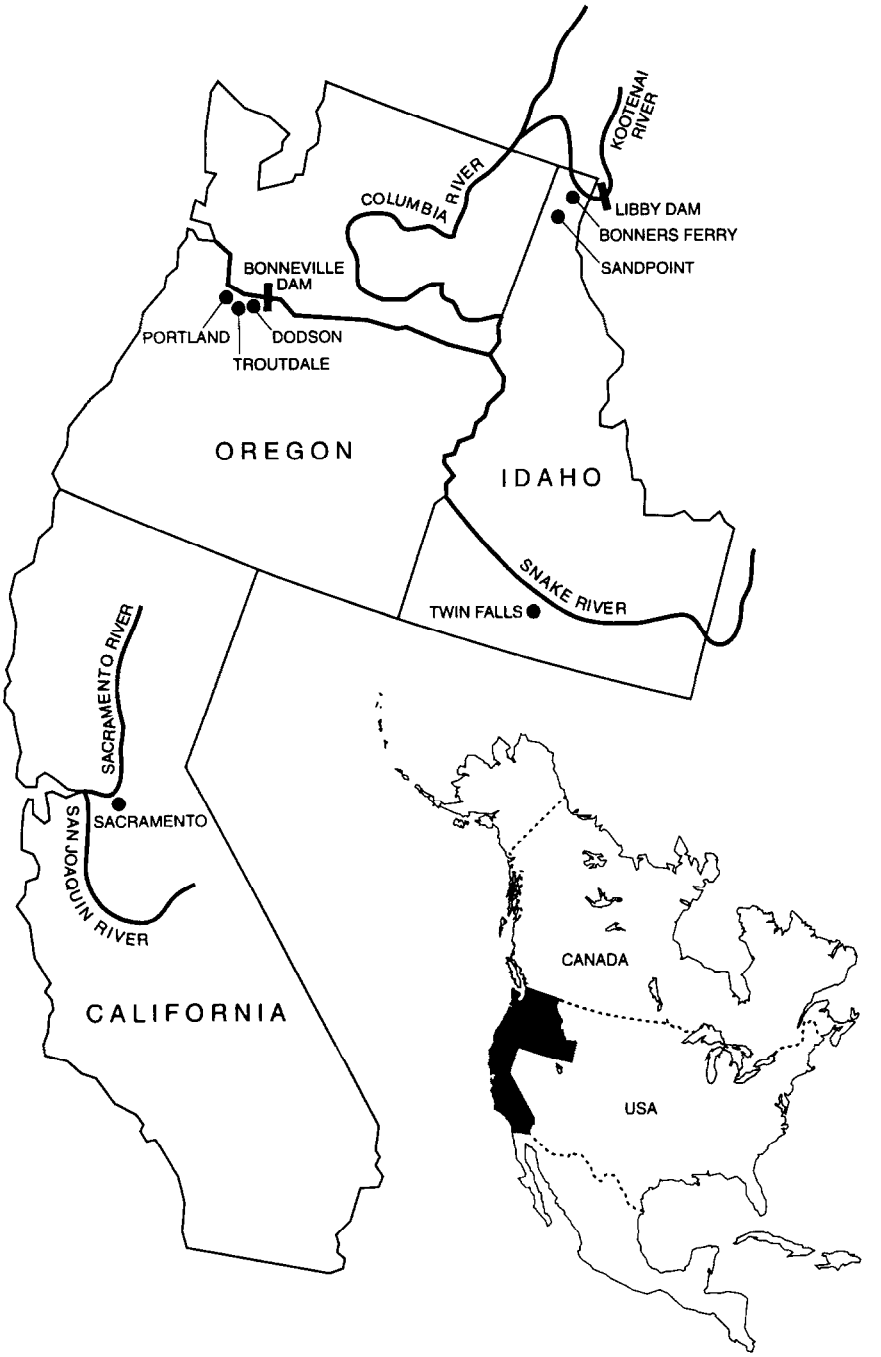

Fig. 1. Map showing location of river systems and hatcheries where juvenile white sturgeon (*Acipenser transmontanus*) were examined for the white sturgeon iridovirus.

artificially spawned at the Dodson facility and three at another private facility at Troutdale, OR, that receives spring water. Some of the sturgeon juveniles from the hatchery at Troutdale were transferred in June to the Dodson facility. Fish culturists at the Dodson facility reported that ichthyophthiriasis, columnaris disease and saprolegniasis were problems in July and again in September, 1990. In early August, tissues from live juveniles from both private hatcheries were submitted for viral isolation. About 900 juvenile sturgeon were transferred to the Oregon Department of Fish and Wildlife Columbia Region headquarters, Clackamas, OR, for further rearing in 12°C spring water. After about a month these juveniles were also tested for virus. Additionally, 30 moribund fish were collected from the Dodson facility in September, 1990 for histopathologic and electron-microscopic examination.

Mid-Snake River, Idaho. In 1987 a cooperative white sturgeon aquaculture program was started in Idaho. Participants in the program included Idaho Department of Fish and Game (IDFG), the commercial aquaculture industry, and College of Southern Idaho (CSI), Twin Falls, ID. The objectives of the program are to provide sturgeon to enhance and expand the resource and to examine the potential of this species in commercial aquaculture. Wild sturgeon adults captured from the Snake River were successfully spawned in 1988, 1990, and 1991. Clinical specimens from the 1991 Snake River white sturgeon brood year were obtained from the CSI Aquaculture Program during November, 1991 when fingerling mortality increased. Additional specimens were obtained from CSI and five commercial producers (CSF, ARK, PS, TS, and FBI) located in the Thousand Springs area of the Hagerman Valley, ID (Fig. 1). All these aquaculture facilities receive spring water supplies with ambient temperatures of 13–15°C. About 6 months after the first examination, 10 additional morbid and 10 apparently healthy appearing sturgeon of the same brood year were collected from CSI, CSF, and ARK for histopathologic examination.

Kootenai River, Idaho. An estimated 880 sturgeon live in the lower Kootenai River in northern Idaho, but they apparently have not reproduced since the Libby Dam was built in western Montana upriver 20 years ago (Apperson and Anders, 1989). A small sturgeon hatchery opened in 1991 near Bonners Ferry, ID, and wild sturgeon adults captured from the Kootenai River were used as broodstock. During November, 1992, mortality in juvenile white sturgeon at the Kootenai Hatchery occurred after a temporary loss of pumped spring water when a portion of the fish were being cultured in dense rearing conditions. About 800 of the affected sturgeon were subsequently transferred to the IDFG Sandpoint Hatchery, also in northern Idaho, to lessen fish densities and improve rearing conditions (Fig. 1). Tissues were collected from moribund fish at both sites and submitted for clinical examination.

Clinical examination procedures

Clinical methods have been described previously (Amos, 1985) or are considered general procedures for routine diagnostic examinations of fish with modifications as indicated below. Briefly, gills, spleen and kidney were tested for bacteria by inoculation of tryptic soy agar and cytophaga agar supplemented with 10% bovine serum and incubated at 15°C. Virology samples included skin, gill and pools of kidney, spleen, and liver tissues that were homogenized 1 : 10 in a balanced saline solution and centrifuged prior to inoculation. Two

cell lines established from white sturgeon (WSS-2 and WSSK-1; Hedrick et al., 1991b) and the CHSE-214 (Lannan et al., 1984) and EPC (Fijan et al., 1983) cell lines were incubated at 18°C and used for primary isolation and subculturing of suspect replicating agents. Tissues to be used for histological examinations were fixed in Bouin's solution for 24–48 h, rinsed and stored in 70% ethanol before paraffin embedding, sectioning and staining (Humason, 1979). Tissue and cell culture specimens for electron microscopy were placed in Karnovsky's fixative for 1 h at room temperature or 2.5% glutaraldhyde in phosphate-buffered saline (PBS), rinsed and stored in PBS at 4°C. Following this, specimens were rinsed twice in buffer and then post-fixed in 1% aqueous OsO_4, dehydrated through a graded ethanol series, infiltrated and embedded in epoxy resin. Thin sections (10–20 nm) were stained with 4% uranyl acetate and lead acetate prior to examination using a Philips EM400 transmission electron microscope.

3. Results

Lower Columbia River, Oregon. Juvenile fish that were transferred to Clackamas, OR, and held for monitoring became weak and emaciated with pale livers and empty intestines. Approximately 25% died after 1 month. All 30 fish collected from the Dodson facility in September had basophilic cells in the epidermis of the integument and gill epithelium pathognomonic of WSIV infection (Hedrick et al., 1990). Additional samples of these tissues observed by electron microscopy had virus particles with a diameter of 267 nm and a morphology typical of WSIV within epithelial cells (Hedrick et al., 1992). Juvenile losses at the Dodson facility were approximately 23% in October, 1990. No cytopathic effect was detected in cell cultures inoculated with tissue specimens from either location.

Mid-Snake River, Idaho. Comparison of daily percent mortality in sturgeon at CSI and CSF showed increased mortality beginning at about 200 days post-hatch in fish at CSI (Fig. 2). Bacteria detected in the specimens obtained from CSI during the increased mortality period included *Aeromonas* sp. and *Pseudomonas* sp. These bacteria were detected in low numbers and were not considered to be the cause of mortality. Subtle cytopathic effects (CPE) were observed after incubation of cells inoculated with tissue homogenates from the same fish incubated for 8 days on the CHSE-214 cell line. Subculturing on the WSS-2 and WSSK-1 cell lines appeared to produce CPE, but this could not be reproduced. No definitive results were obtained after an electron microscopic examination of these suspected virus-infected cell cultures. Histologic examination of fish from CSI exhibited lesions typical of white sturgeon iridovirus (WSIV) infection (Fig. 3). Electron-microscopic examination of sturgeon tissue, obtained from fish after the increased mortality had subsided, yielded no definitive evidence of iridovirus infection. However, tissues from fish at CSI used for the original histological diagnosis were deparaffinized and processed for electron-microscopic examination and did reveal the presence of iridovirus-like particles, approximately 270 nm in diameter, within epithelial cells of the integument and gills (Fig. 4). Sturgeon obtained from two commercial producers, ARK and PS, were also diagnosed histologically as WSIV-positive. Seventy-one fish were examined from CSF at various stages of development and WSIV was not detected (Table 1). No histological evidence of WSIV infection was

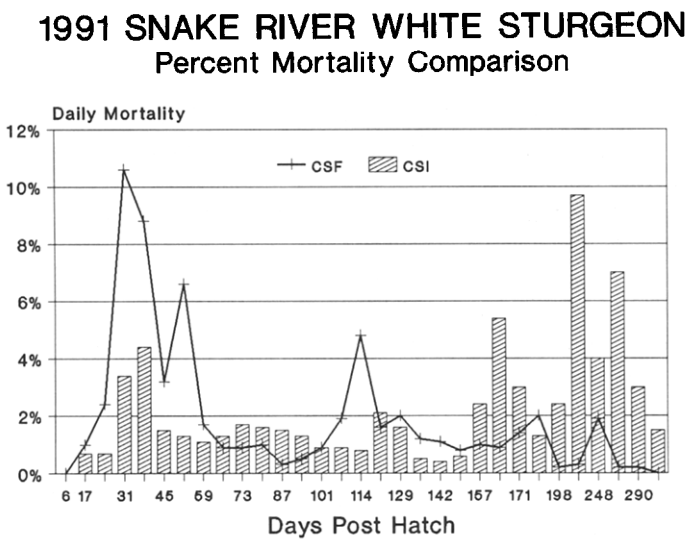

Fig. 2. Daily percent sturgeon mortality in 1991 broodyear Snake River white sturgeon (*Acipenser transmontanus*) at College of Southern Idaho (CSI) Aquaculture Program, Twin Falls, ID, and Clear Springs Foods (CSF), Inc., Buhl, ID, USA.

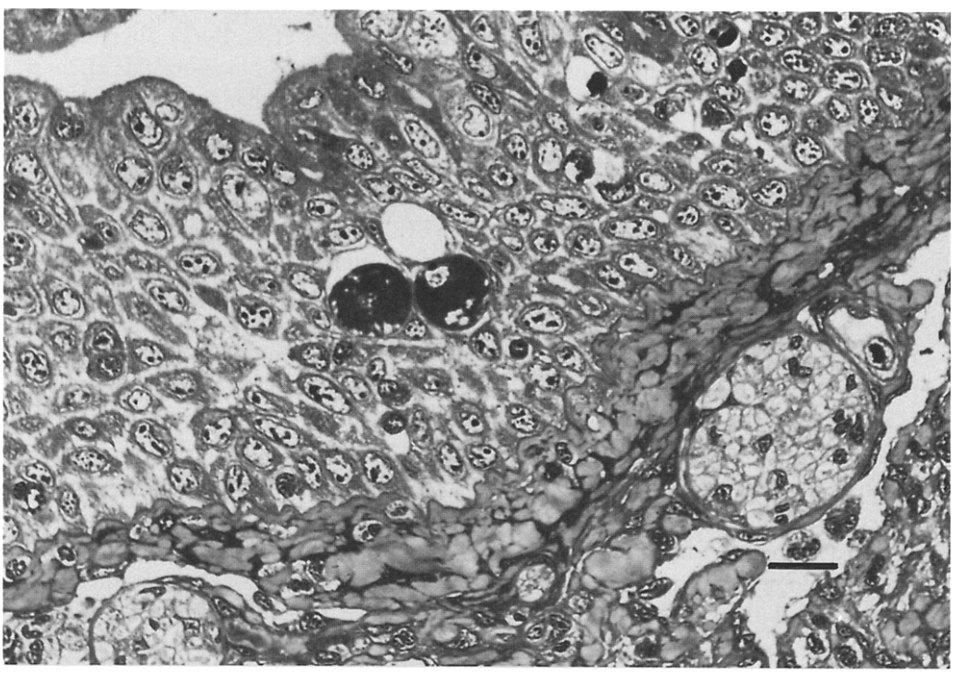

Fig. 3. Semi-thin gill tissue section from white sturgeon *Acipenser transmontanus* stained with toludine blue. Enlarged and deeply stained cells in the epithelium of the gills (×600). Bar = 20 μm.

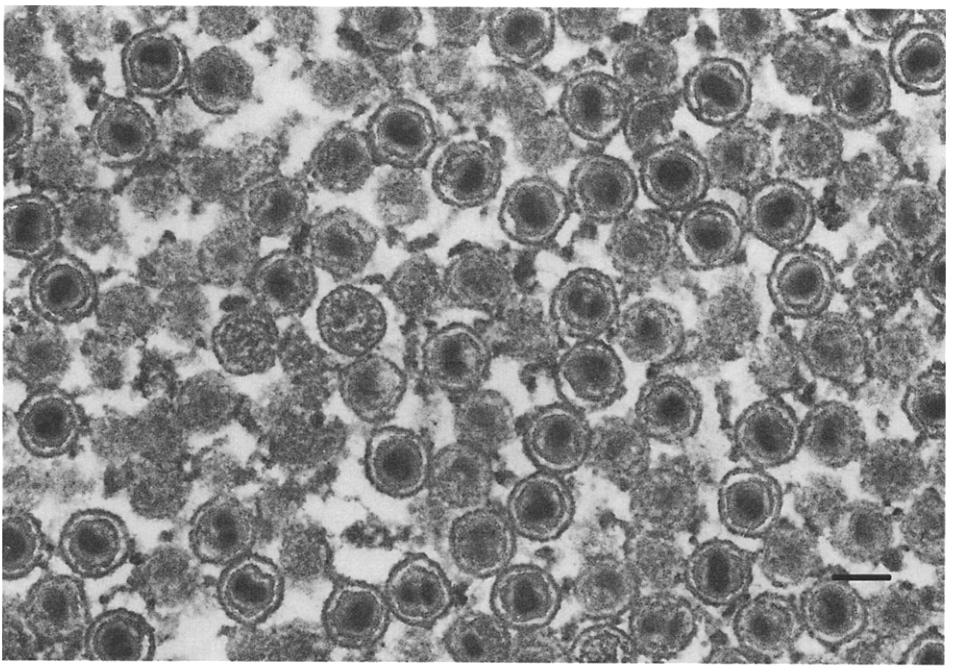

Fig. 4. Iridovirus-like particles in the epithelial cells of gill tissue from white sturgeon *Acipenser transmontanus*. Lead citrate-uranyl acetate; ×50 000. Bar = 200 nm.

Table 1
Total number of 1991 broodyear Snake River white sturgeon (*Acipenser transmontanus*) examined by histological methods and number positive for the white sturgeon iridovirus (WSIV) in fish obtained from College of Southern Idaho (CSI) Aquaculture Program, Clear Springs Foods (CSF), Inc., Babbington Enterprises (ARK), and Pristine Springs (PS)

Location	Total fish examined	Percent (proportion) with signs of WSIV
CSI	26	88 (23/26)
CSF	71	0 (0/71)
ARK	12	92 (11/12)
PS	10	30 (3/10)

observed in yearling sturgeon obtained from CSI and ARK 6 months after the original diagnosis.

Kootenai River, Idaho. Microscopic examination of epithelial tissues from fish at both the Kootenai and Sandpoint hatcheries revealed lesions typical for WSIV infection. Subsamples from fish at the Kootenai Hatchery used for the original histological diagnosis were deparaffinized and processed for transmission electron microscopy. Examination of these specimens revealed the presence of iridovirus-like particles typical of WSIV. Of the affected sturgeon transferred from Kootenai to Sandpoint, 75% died compared to the 48% loss at

Kootenai. The stress of transporting may have exacerbated mortality at Sandpoint. A total of 52% (2600/5000) fish died as a result of the epizootic.

4. Discussion

The source of WSIV that infected juvenile sturgeon being cultured at hatcheries in the Lower Columbia River, Snake River, and Kootenai River is unknown. In California, WSIV has been detected at several commercial aquaculture farms where the original source of virus is believed to be from wild sturgeon adults held for broodstock.

In Oregon, only the hatchery at Dodson supplied with Columbia River water had sturgeon detected as WSIV-positive. Fish at the hatchery at Troutdale, that received spring water, remained virus-free and did not experience elevated mortality. Infected wild sturgeon in the Columbia River may have been the source of WSIV that infected juvenile sturgeon being reared in river water.

In southern Idaho, the CSI program has held 2–6 wild adult sturgeon obtained from the Snake River on site each year since 1987. These may have been the source of infection for the juvenile fish being reared at CSI. Gill specimens obtained non-lethally from 12 wild adult sturgeon caught from the Snake River were examined and showed no evidence of WSIV infection (Keith Johnson, Idaho Department of Fish and Game, personal communication). Evaluations and conclusions about wild adult fish are limited by the number of fish sampled, the need to use specimens that can be obtained non-lethally, precision of the diagnostic techniques available, and lack of knowledge about the virus. Possibly, when more fish are examined using more sensitive diagnostic techniques, asymptomatic adult sturgeon with WSIV will be detected.

Sturgeon that tested WSIV-positive at a private facility in southern Idaho (ARK) were received from CSI in October, 1992. We suggest the virus may have been transferred to this area with the movement of these sturgeon. Another commercial producer, CSF, received 4000 larval sturgeon from the same mating pair crosses as the fish at CSI and ARK, but no WSIV was detected. A possible explanation for WSIV not being detected at CSF may be the limited number and sensitivity of diagnostic procedures available, different fish culture conditions, and the fact that CSF has never had any wild-caught Snake River adult sturgeon on site.

Another commercial producer (PS) that received larvae from the same mating cross as the other commercial producers also had fish diagnosed with WSIV. These sturgeon were cultured in a spring water supply and no adult sturgeon had ever been held at this hatchery. The affected sturgeon were small and emaciated and egg-associated transmission of WSIV may have occurred from broodstock obtained from the wild. In California, disinfection of fertilized eggs in iodophor solutions has been examined as a management strategy to prevent or minimize WSIV egg-associated transmission, but the results have been equivocal.

Characteristics of the Kootenai epizootic appeared similar to those reported for cultured juvenile sturgeon in southern Idaho. All egg incubation and rearing of larval and juvenile sturgeon at the Kootenai Hatchery prior to the epizootic had been on spring water. Mortality began in 6-month-old sturgeon, 10–15 cm length, after periods of low water flows when fish densities were high. When densities were reduced and water flows increased, mortality

subsided. These observations indicate that infection may be present, but disease does not occur until the sturgeon are compromised or stressed. This is supported by the lack of mortality in other groups of juvenile white sturgeon from the same source held at Kootenai Hatchery which had their densities reduced prior to low flows.

Additionally, sibling juvenile sturgeon that were reared since the fertilized egg stage under conditions relatively free of stress at an IDFG hatchery in the same area (Sandpoint Hatchery; Fig. 1) exhibited no mortality. Sturgeon exposed to WSIV and transferred to Sandpoint after the epizootic began at Kootenai Hatchery, also died. Since this disease appears size(age)-specific and stress-mediated, fish culture management strategies could be used to minimize epizootics. These would include maintaining low fish densities and loadings, minimizing adverse environmental conditions (e.g. water failure), and reducing the handling of fish younger than 1 year.

We suspect that in each case described in this report the source of virus infecting and causing mortality in cultured juvenile sturgeon was from wild sturgeon. The virus may have come into the facility by waterborne virus from fish present in the river or by egg-associated transmission from fish caught from the rivers and used for broodstock. WSIV appears to be enzootic in the Sacramento-San Joaquin, Columbia, Snake, and Kootenai Rivers. If the virus does occur in wild white sturgeon, it may be present in most Northwest populations due to the long life span of the species, migratory patterns, and continuity of the river systems. White sturgeon are known to migrate from one river system to another by entering the Pacific Ocean (Chadwick, 1959). Since 1986, at least three white sturgeon tagged in the Sacramento-San Joaquin systems have subsequently been captured in the Lower Columbia River (Steve D. King, ODFW, Columbia River Management, Clackamas, OR). Continued monitoring of wild and hatchery-reared sturgeon will be required before any conclusions can be made relative to adult white sturgeon serving as reservoir hosts to WSIV.

Acknowledgements

The authors wish to thank Kathy Lauda, Clear Springs Foods (CSF), Inc., Buhl, ID; Harriet Lorz, Department of Microbiology, Oregon State University, Corvallis, OR; John Kaufman, Oregon Department of Fish and Wildlife (ODFW), Corvallis; and Terry McDowell, Department of Medicine, School of Veterinary Medicine, University of California, Davis, CA (UCD) for the tissue culture work. Histological preparatory work was done by Roberta Scott and Carla Hogge, Idaho Department of Fish and Game (IDFG), Eagle, ID. Specimen collections were done by Rich Schneider, CSF, Terry Kreps, ODFW, Clackamas, OR, and Jack Siple, IDFG, Bonners Ferry, ID. Electron-microscopy assistance was provided by Vern Winston, Department of Biological Sciences, Idaho State University, Pocatello, ID.

This paper is funded in part by a grant from the National Sea Grant College Program, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, under grant number NA89AA-D-SG138, project number R/A-89 through the California Sea Grant College, and in part by the California State Resources Agency.

The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its sub-agencies. The U.S. Government is authorized to reproduce and distribute for governmental purposes.

References

Amos, K., 1985. Procedures for the detection and identification of certain fish pathogens, 3rd edn. American Fisheries Society, Fish Health Section, Corvallis, OR.

Apperson, K.A. and Anders, P.J., 1989. Kootenai River white sturgeon investigations and experimental culture. Annual progress report, funded by Bonneville Power Administration, Division of Fish and Wildlife, Portalnd, OR. Contract No. DE-AI79-88BP93497.

Chadwick, H.K., 1959. California sturgeon tagging studies. California Fish Game, 45: 297–301.

Fijan, N., Sulimanovic, D., Bearzotti, M., Munzinic, D., Zwillenberg, L.D., Chilmonczyk, S., Vautherot, J.F. and de Kinkelin, P., 1983. Some properties of the *Epithelioma papulosum cyprini* (EPC) cell line from carp *Cyprinus carpio*. Ann. Virol. (Inst. Pasteur), 134: 207–220.

Hedrick, R.P., Speas, J., Kent, M.L. and McDowell, T.S., 1985. Adenovirus-like particles associated with a disease of cultured white sturgeon, *Acipenser transmontanus*. Can. J. Fish. Aquat. Sci., 42: 1321–1325.

Hedrick, R.P., Groff, J.M., McDowell, T.S. and Wingfield, W.H., 1990. An iridovirus infection of the integument of the white sturgeon *Acipenser transmontanus*. Dis. Aquat. Org., 8: 39–44.

Hedrick, R.P., McDowell, T.S., Groff, J.M., Yun, S. and Wingfield, W.H., 1991a. Isolation of an epitheliotrophic herpesvirus from white sturgeon *Acipenser transmontanus*. Dis. Aquat. Org., 11: 49–56.

Hedrick, R.P., McDowell, T.S., Rosemark, R., Aronstein, D. and Lannan, C.N., 1991b. Two cell lines from white sturgeon. Trans. Am. Fish. Soc., 120: 528–534.

Hedrick, R.P., McDowell, T.S., Groff, J.M., Yun, S. and Wingfield, W.H., 1992. Isolation and properties of an iridovirus-like agent from white sturgeon *Acipenser transmontanus*. Dis. Aquat. Org., 12: 75–81.

Humason, G.L., 1979. Animal Tissue Techniques. W.H. Freeman Co., San Francisco.

Lannan, C.N., Winton, J.R. and Fryer, J.L., 1984. Fish cell lines: establishment and characterization of nine cell lines from salmonids. In Vitro, 20: 671–676.