

An iridovirus infection of the integument of the white sturgeon *Acipenser transmontanus*

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ABSTRACT: A newly recognized iridovirus was found to be infecting the integument of the juvenile white sturgeon *Acipenser transmontanus*. Only epithelial cells in the skin and gills were infected. Infected cells were enlarged and often very basophilic. Virions with a mean diameter of 262 nm were found within infected cells. In heavy infections, the skin and gills of dead and moribund fish showed numerous infected cells. No other pathogens were detected in sturgeon that succumbed to the disease, and it is likely that the virus was the cause of the mortality. A laboratory study indicated that the virus could be transmitted via water to uninfected white sturgeon held below infected fish.

INTRODUCTION

The artificial culture of white sturgeon *Acipenser transmontanus* has been steadily growing in California, USA, since 1978. Presently, 12 farms are actively engaged in rearing sturgeon from the spawning of feral broodstocks. Growth rates obtained in captivity have been exceptional, and these fish have been marketed both for the aquarium trade and as food fish. Broodstock development and diseases encountered in the early-rearing phases are the major difficulties now encountered by this industry.

The principal disease problems during egg incubation are associated with fungal infections. In juveniles, diseases associated with adaptation to artificial diets are believed to contribute to poor survival. During this period, juvenile sturgeon can experience bacterial gill infections, liver diseases of unknown etiology, and adenovirus infections of the alimentary tract (Hedrick et al. 1985). Generally, after fish have reached 15 cm in length, few problems are encountered. For the past 2 yr however, juvenile fish (usually less than 15 cm, but in some situations up to 25 cm in length) at several farms have suffered from infections of the skin and gills that have resulted in substantial mortality. In our light and electron microscopic examinations of these tissues we have shown these infections to be associ-

ated with the presence of a previously unknown iridovirus.

Six iridoviruses have been isolated from fish and 2 others observed by electron microscopy (Wolf 1988). Two, possibly 3 viruses, are associated with infections of the integument. The first to be described and isolated was lymphocystis virus, a cause of cellular hypertrophy in the integument of many species of freshwater and marine fish (Walker 1962, Wolf et al. 1966). The second iridovirus was associated with the skin of Atlantic cod, *Gadus morhua*, with ulcer disease (Jensen et al. 1979). The third iridovirus, isolated from diseased eels, *Anguilla japonica*, from Japan (Sorimachi & Egusa 1982) may also be associated with infection of the integument (Sorimachi 1984).

Additional iridoviruses isolated from redbfin perch (*Perca fluviatilis*), common carp (*Cyprinus carpio*), and goldfish (*Carassius auratus*) are associated with diseases that range from fatal systemic conditions to inapparent infections (Shchelkunov 1978, Shchelkunov & Shchelkunova 1981, 1984, Berry et al. 1983, Langdon & Humphrey 1987, Langdon 1989).

A group of iridoviruses observed in the erythrocytes of many marine or anadromous fishes are the cause of viral erythrocytic necrosis or VEN (Evelyn & Traxler 1978, Smail 1982). They are associated with anemia in fish from North America, Greenland, the United Kingdom, and the Mediterranean (Wolf 1988).

The most recently described iridovirus is associated with a systemic disease in chromide cichlids, *Etropus*

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maculatus, imported into Canada (Armstrong & Ferguson 1989). The agent, however, has yet to be isolated in cell culture.

These reports show that iridoviruses cause a spectrum of syndromes ranging from fatal systemic disease to benign, inapparent infections. In this paper we describe a newly recognized virus from the integument of white sturgeon, and present microscopic evidence that suggests that this iridovirus can cause serious damage to the skin and gills of infected sturgeon. The rapid spread of the virus among populations of fish at several sturgeon farms in California was a major obstacle to production in 1988. In one farm with 200 000 juvenile sturgeon, 95 % of the fish died over a 4 mo period. Oral and bath treatments with antibiotics, and immersion in external parasiticides failed to influence mortality. Over the 4 mo period, numerous fish were submitted to the laboratory and no other causes of mortality other than the virus were detected.

MATERIALS AND METHODS

Fish. Virus-infected juvenile white sturgeon (2 to 10 g) were obtained from 2 commercial farms. These infected sturgeon and a laboratory-reared, virus-free group of juvenile white sturgeon (2 to 6 g) were used for the transmission study. Groups of 10 fish from the virus-free control population were examined at 1 and 2 mo of age and at the initiation and termination of the transmission trial, by histological methods to determine whether the iridovirus was present.

Light and electron microscopy. Sturgeon less than 5 g in size were fixed, whole, in Davidson's solution (Humason 1979) for 24 h and then transferred to 70 % ethanol. Portions of the gill and skin (of the operculum of larger fish) were fixed in the same manner. Whole fish or individual tissues were embedded in paraffin, sectioned to 5 μm , and stained with hematoxylin and eosin (H & E).

Tissues for electron microscopy were fixed in 2.5 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4), post-fixed in 1 % aqueous OsO_4 , embedded in plastic, and sectioned. Sections were stained with uranyl acetate and lead citrate, examined and photographed using a Philips EM 400 electron microscope.

Transmission trials. Two sturgeon from a population, shown to be infected with the iridovirus by histological and electron microscopical examinations of the gills, were placed in the upper section of a 70 l trough with running 17°C well-water. In the lower section of the trough, separated from the upper section by a screen, 10 healthy sturgeon were added. In a second identical trough, the upper section was left empty, while the lower section contained 10 healthy sturgeon.

Stained sections of the gill and skin were examined by light microscopy from fish that died in any of the groups. After 40 d, all surviving fish were sacrificed and examined for evidence of viral infections using the histological methods described for dead fish.

RESULTS

Gross signs of disease

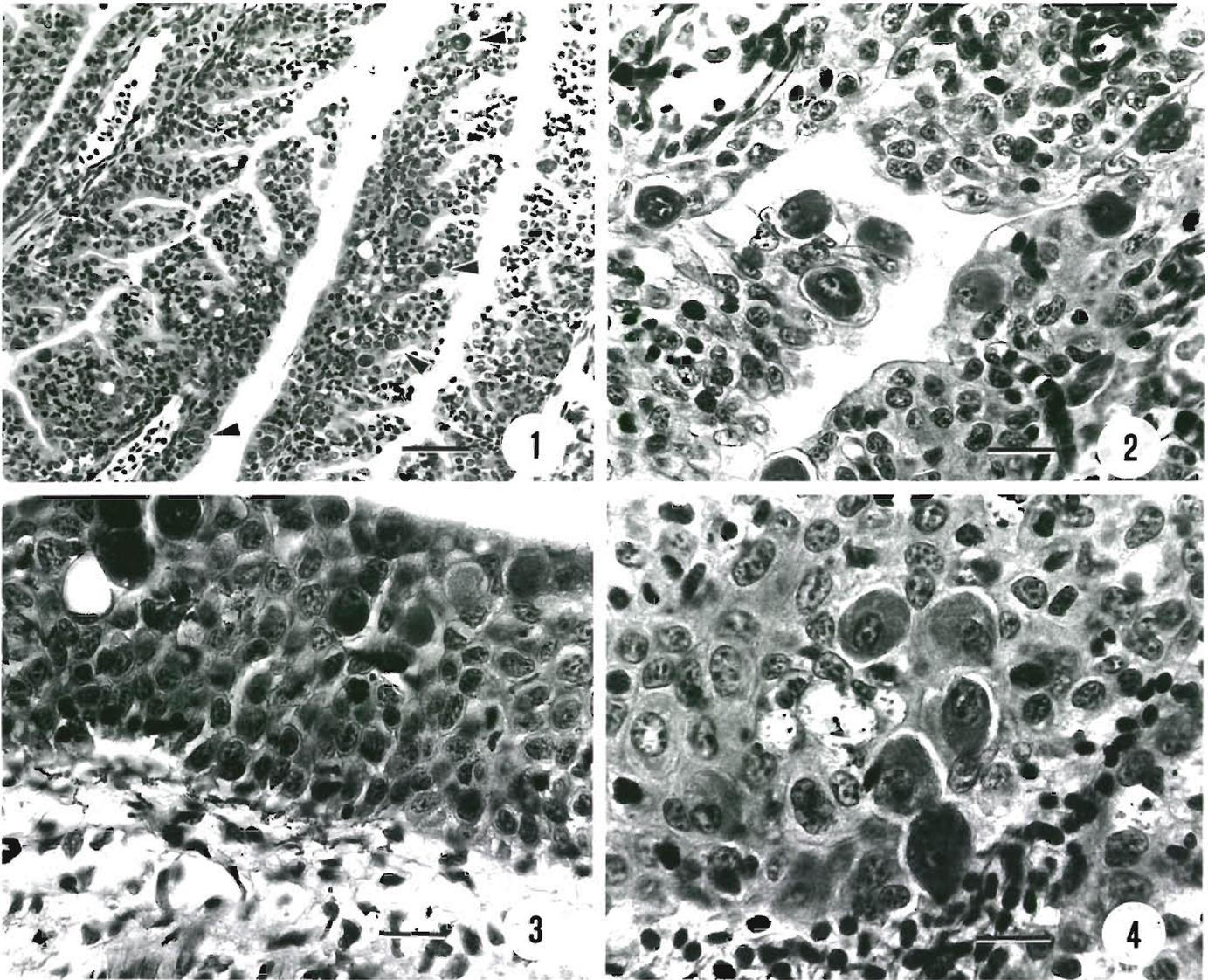
In outbreaks at commercial farms and in the laboratory transmission trial, juvenile sturgeon with iridovirus infections proved to be the weaker fish in the population and exhibited weight loss. Affected fish dropped to the bottom of the tank, ceased swimming, and eventually died. Gill pallor was also evident. Internally, fish had little to no body fat and pale livers; in addition, the gastrointestinal tract was empty.

Microscopic signs of disease

Histological examination of the gills and skin showed numerous hypertrophic, and occasionally intensely basophilic, cells in the epithelium and epidermis (Figs. 1 to 4). Crystalline rod-like bodies were observed in the cytoplasm of some infected cells, and the nucleus was often enlarged (Fig. 4). Cells in proximity to the heavily stained cells were also affected, as shown by nuclear swelling and cell enlargement (Fig. 4).

Nearly all Malpighian cells of the epidermis of some fish were infected, as judged by their appearance on light microscopy. In these fish, the epidermis could be seen separating from the underlying dermis (not shown). More advanced gill lesions developed in some fish. These included hyperplasia of the respiratory epithelium followed by necrosis of the pillar cells lining the lamellar vascular channels. Small hemorrhages were often associated with these vascular lesions. Hypertrophic, basophilic cells were observed but less frequently in these fish.

Enlarged cells containing numerous virions were seen on electron microscopy of gill tissues (Fig. 5). These cells were often more electron dense and appeared to be in the process of separating from adjacent epithelial cells. Spaces containing cell remnants were presumed to be locations where virus-infected cells had lysed (Fig. 5). Virions were abundant in the cytoplasm of degenerating cells near the epithelial surface, and accumulations of fibrillar material were present (Fig. 6). Cells adjacent to those containing complete virions contained immature forms of the virus in their cytoplasm. Complete virions were 262 nm ($n = 20$, $\text{SD} = 7.0$ nm) in diameter as measured from



Figs. 1 to 4. *Acipenser transmontanus*. Specimen infected with the white sturgeon iridovirus and examined by light microscopy using H & E. Fig. 1 (bar = 100 μ m) and Fig. 2 (bar = 10 μ m) Enlarged and deeply stained cells in the epithelium of the gills. Fig. 3. Affected cells in the epidermis. Bar = 10 μ m. Fig. 4. Affected cells in the gill showing nuclear abnormalities and crystalline rod-like bodies in the cytoplasm. Bar = 10 μ m

one side to another and 299 nm from vertex to vertex (Fig. 7). There was an internal nucleoid that surrounded a circular to bar-shaped electron dense core (Fig. 7). This internal capsid or nucleoid had a diameter of 184 nm ($n = 10$, $SD = 11.5$ nm).

Transmission trial

Infections were detected in white sturgeon that were held below infected sturgeon but not in control fish. One of the 2 infected sturgeon, used as the virus source, died 2 d after initiation of the study. Histological examination of the skin and gills of this fish showed that it was heavily infected, as determined by the

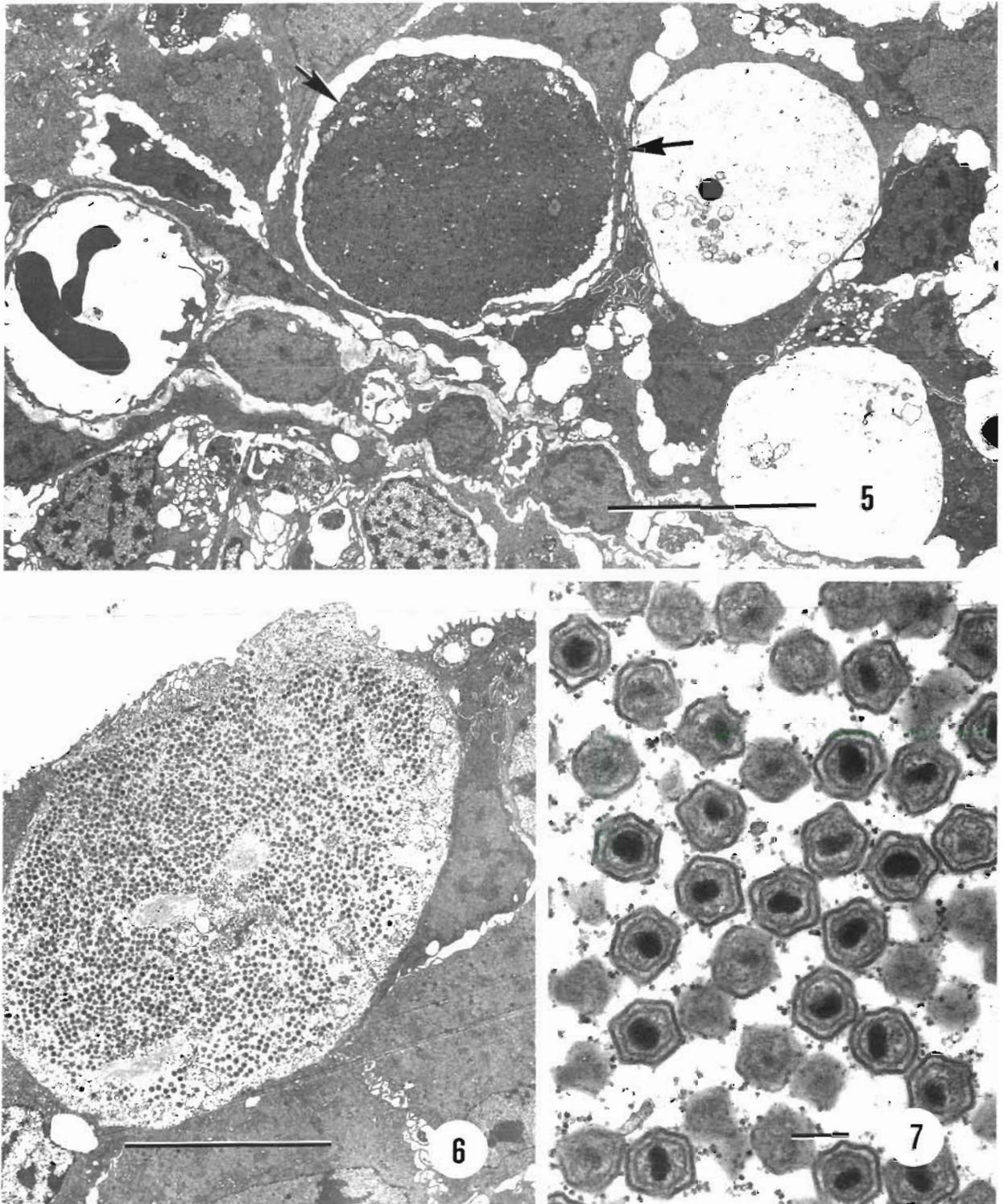
presence of large numbers of hypertrophied, basophilic cells. Four of the 10 sturgeon exposed to infected fish died by 40 d postexposure. Evidence for virus infection was found on histological examination in 2 of these fish and in 4 of the 6 fish that were sacrificed at the end of the study (40 d). No control fish died during the study and there was no histological evidence of infection in these fish when they were examined at the end of the study.

DISCUSSION

The white sturgeon iridovirus (WSIV), described in this study, displayed a tropism for cells of the epidermis

and infection of these cells ultimately led to their lysis. Involvement of substantial portions of the epidermis was the likely cause of death in affected fish. In midline

sagittal sections of whole small fish, there was no evidence of WSIV infection in any of the other tissues examined.



Figs 5 to 7 *Acipenser transmontanus* Specimen infected with the white sturgeon iridovirus and examined by electron microscopy Fig. 5. Infected epithelial cells in the gill. Arrows show an electron dense cell containing virions. Bar = 10 μ m. Fig. 6. Virus infected cell rupturing to the exterior. Bar = 10 μ m. Fig. 7. Virions with nucleoids. Bar = 20 nm

Electron microscopy confirmed the epithelial nature of the virus-infected cells in the gill (Figs. 5 to 7). Although hypertrophy was a feature of infected cells, this was less pronounced than that resulting from infection with the lymphocystis virus which causes marked enlargement of fibroblasts (Wolf et al. 1966). In contrast to lymphocystis virus infections where nuclear but not cytoplasmic divisions occur, the fate of WSIV-infected cells in sturgeon is assumed to be lysis (Fig. 6). Prior to lysis, the cells fill with completely formed virions and the cell cytoplasm loses all normal structure. These cells then lyse and detach from adjacent cells, leaving spaces in the tissue (Fig. 5).

Affected cells exhibited swelling of the nucleus and cytoplasm and ranged in their staining properties from almost normal to intensely basophilic. The latter cells appear to be pathognomonic for this infection as we have not seen similar changes in normal sturgeon, or in sturgeon infected with other pathogens. A correlation between the presence of these cells in the skin and gill with the occurrence of the virus was noted in 2 separate outbreaks of the disease. The cellular changes and staining properties of infected cells may correspond to the complex stages associated with iridovirus replication, which involves both nuclear and cytoplasmic phases. Initially, viral DNA replication occurs in the nucleus (Stage 1). A second and more intense DNA replication in the cytoplasm follows (Stage 2) where the concatemeric DNA is cleaved and packaged into virions at assembly sites (Murti et al. 1985). The later phases of viral replication and synthesis in the host cell may give the infected cell the basophilic and electron dense staining characteristics observed on light and electron microscopy, respectively (Figs. 4 and 5).

The shape and structure of fish iridoviruses are similar, but there are wide variations in the virion size even among viruses within the same category. For example, virions of erythrocytic necrosis virus (ENV) in Atlantic herring, *Clupea harengus harengus*, are 146 nm wide, while those in Atlantic cod *Gadus morhua* measure up to 360 nm (Appy et al. 1976, Reno et al. 1978). Sturgeon virus particles are intermediate in size when compared to other fish iridoviruses; however, the nucleoid of the sturgeon virus differs from those of other reported fish iridoviruses. The bar-shaped electron dense core seen in the nucleoid of particles of WSIV contrasts with the circular or hexagonal core of other fish iridoviruses. In addition, the fibrous layer that surrounds many lymphocystis viruses (Yamamoto et al. 1976) was not observed in our WSIV preparations.

The disease caused by WSIV can be severe in young white sturgeon. In one commercial farm, 95% of 200 000 juveniles were lost over a 4 mo period. Large numbers of infected cells were present in the gills and skin of dead and moribund fish from this site and there

was no evidence of other causes of mortality. Concurrent infections of the skin and gills could compromise the osmoregulatory capabilities of affected sturgeon. These changes to the skin, if severe, could cause death without systemic spread of the virus. The severity of these changes to the integument in certain fish was demonstrated by separation of the epidermis from the underlying dermis. In over 100 fish examined, we found no evidence of infections in any internal organs.

We were unable to isolate the sturgeon iridovirus despite numerous attempts involving inoculation of white sturgeon and other fish cell lines. Several other cell-specific iridoviruses, such as ENV and the marine fish lymphocystis viruses, have also not been isolated in cell cultures. Descriptions of the biochemical and serological properties of WSIV and details of its replication will only be forthcoming if the virus can be propagated in cell culture.

The source of WSIV is unknown, but presumably it was introduced to the sturgeon farms with the wild broodstock now used by the industry. We have yet to detect the virus in feral juveniles, and the potential impact of the virus on these fish is currently unknown.

Acknowledgements. This work was supported in part by Wallop-Breaux-Dingell-Johnson Fish Restoration Act Funds provided by the California Department of Fish and Game. Thanks to the commercial aquaculturists who provided fish for examination and Dr A. G. Zapata, Ms K. D. Arkush, and the histotechnicians at the Veterinary Medicine Teaching Hospital for their advice and technical assistance. We are also grateful to Mr R. Munn for the electron microscopy.

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Responsible Subject Editor: Dr T. Evelyn, Nanaimo, B. C., Canada

Manuscript first received: May 8, 1989
Revised version accepted: October 17, 1989