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NEW VIRUSES DESCRIBED IN FINFISH FROM 1988-1992

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Abstract. A number of new virus isolates from finfish have been reported in the scientific literature during the past five years. These include nine aquareoviruses, eight picornaviruses, six iridoviruses, five herpesviruses, three rhabdoviruses, three retroviruses, a paramyxovirus, and a coronavirus. Not all of these agents have been isolated in cell culture or established as etiologic agents of disease by controlled transmission studies. The burgeoning number of fish viruses is a reflection of the increased interest in fish diseases, particularly those occurring in aquaculture facilities, and the number will surely grow as fish farming intensifies on a global scale. This review chronicles the new virus isolates and lists them with other members of their virus family where appropriate.

Keywords. Fish diseases, Fish viruses, Aquareoviruses, Iridoviruses

INTRODUCTION

The book *Fish Viruses and Fish Viral Diseases*, written by Wolf and published in 1988, is the definitive reference work on viral diseases of fish (1). However, during the five years since its publication, more than 35 new viruses have been isolated or identified from finfish. Some of these agents are known to be new agents, while others may simply be isolates of known agents from new hosts. This increase in the number of known fish viruses is a direct reflection of the increased surveillance of fish populations that has accompanied the worldwide expansion of aquaculture. These newly described agents are broadly distributed among the known virus families and include members of the *Herpesviridae*, *Picornaviridae*, *Reoviridae*, *Retroviridae*, *Coronaviridae*, *Iridoviridae*, *Rhabdoviridae*, and *Paramyxoviridae*. Although the focus of this review is on those viruses described during the past 5 years, we have also listed in tabular form the previously described members of the rhabdovirus, herpesvirus, iridovirus, and aquareovirus groups in the hope that such listings will be useful to the reader.

RNA VIRUSES

Reoviridae

During the past decade a number of viruses belonging to the family *Reoviridae* have been isolated from a variety of aquatic animals including finfish, shellfish, and crustacea. These agents have been isolated from wide geographic areas, sometimes from normal animals undergoing routine examination (2). The International Committee for the Nomenclature of Viruses has recently approved the genus name *Aquareovirus* (3), suggested by Winton (4), for the aquatic reoviruses bringing the number of genera in the family *Reoviridae* to eight.

New aquareoviruses. Table 1 lists the reoviruses described in finfish including the nine new isolations made during the last five years from different finfish species.

Winton et al. (11) described a reovirus (CSR) recovered from adult coho salmon (*Oncorhynchus kisutch*) and chinook (*O. tshawytscha*) salmon returning to three locations in Oregon. The virus was not neutralized by antiserum against chum salmon virus (CSV), the only other salmonid reovirus known at the time. A second and potentially related salmonid reovirus has been isolated from adult chinook salmon in Northern California but its relationship to CSR and CSV is currently unknown. Experimental infection of rainbow trout with CSR did not result in mortalities or overt disease but virus was detectable in the fish throughout the 11-week period of the experiment. There were necrotic lesions in the liver produced by viral replication.

Another reovirus-like agent was isolated from landlocked salmon (*Oncorhynchus masou*) in Taiwan by Hsu et al. (12). The isolate not only showed the typical syncytial type of cytopathic effect (CPE) in CHSE cells but it also replicated well in bluegill

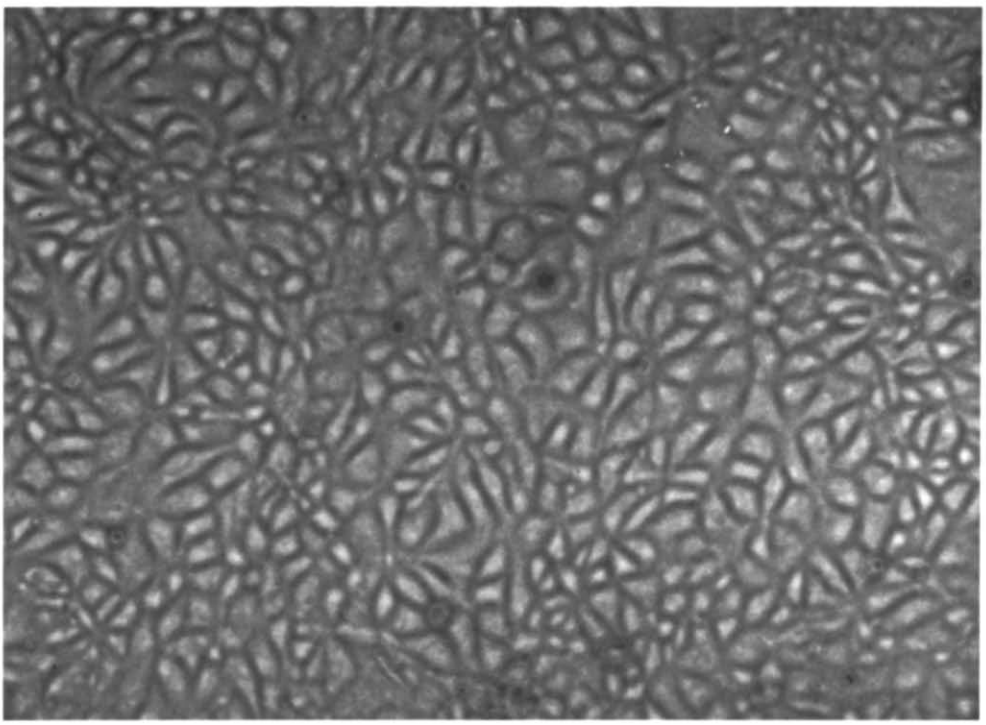
Table 1. Aquareoviruses isolated from finfish

Virus	Naturally infected host	Geographic distribution	Source tissue	Disease	Reference
		Prior to 1988			
Golden shiner (GSV)	*Notemigonus crysoleucas*	Southern USA	Viscera	Yes	(5)
Chum salmon (CSV)	*Oncorhynchus keta*	Japan	Kidney/spleen	No	(6)
Catfish reovirus (CRV)	*Ictalurus puncatatus*	Western USA	Systemic	No	(7)
Grass carp reovirus	*Ctenopharyngodon idella*	China	Systemic	Yes	(8)
Tench reovirus	*Tinca tinca*	Germany	Viscera	No	(9)
Chub reovirus	*Leuciscus cephalus*	Germany	Viscera	No	(9)
Fancy carp	*Cyprinus carpio*	Japan	Kidney/spleen	No	(10)
Japanese eel	*Anguilla japonica*	Japan	Viscera	No	(10)
		From 1988–1992			
Turbot (TRV)	*Scophthalmus maximus*	Spain	Viscera	No	(20)
Landlocked salmon (LSV)	*Oncorhynchus masou*	Taiwan	Viscera	No	(12)
Coho salmon (CSR)	*Oncorhynchus kisutch*	Western USA	Viscera	No	(11)
Smelt (SRV)	*Osmerus mordax*	Canada	Viscera	Yes	(18)
Striped bass (SBR)	*Morone saxatilis*	Eastern USA	Kidney, spleen	No	(16)
Atlantic salmon (ASR)	*Salmo salar*	Canada	Ovarian fluid	No	(17)
Common carp	*Cyprinus carpio*	China	Viscera	No	(14)
Angelfish	*Pomacanthus semicirculatis*	USA	Liver, spleen	Yes	(13)
Grouper	*Plectropomus maculatus*	Singapore	Liver, spleen	Yes	(15)

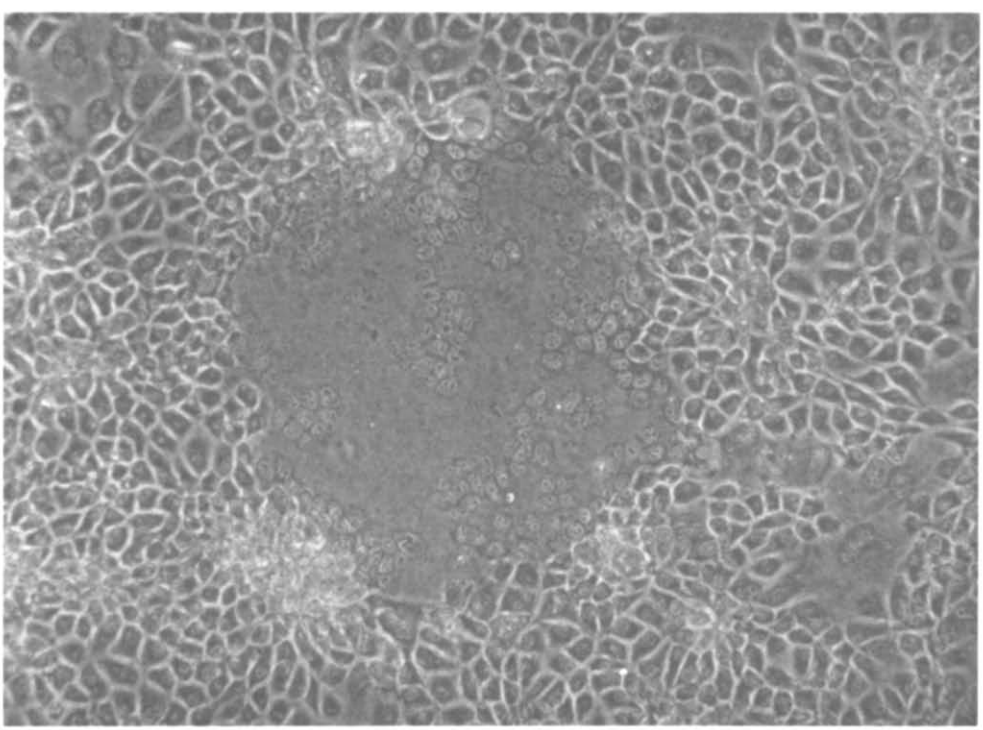
fry (BF-2), brown bullhead (BB), channel catfish ovary (CCO), and Atlantic salmon (AS) cells, the optimal growth temperature being 20 °C. Varner and Lewis (13) isolated a reovirus from marine angelfish (*Pomecanthus semicirculatis*) exhibiting head and lateral line erosion (HLLE) syndrome. Superficial erosions of the head and face progress down the lateral flank to the lateral line. Healthy angelfish exposed to the isolate died after developing similar lesions. Jiang and coworkers (14) in the People's Republic of China reported the isolation of a reovirus from the internal organs of common carp (*Cyprinus carpio*) with epidermal hyperplasia. The virus grows in cell lines of both common carp and grass carp origin and produces the characteristic syncytia that are the hallmark of these agents. Attempts to experimentally infect common, grass, silver, or bighead carp were all unsuccessful. Most recently, Chew-Lim et al. (15) isolated a virus from the spleens of sick grouper (*Plectropomus maculatus*) being cultured in Singapore. The virus was isolated in a cell line derived from Asian seabass fry but it also grew in BF-2 cells. A limited characterization of the agent indicated properties similar to those of the aquareoviruses, particularly the above mentioned carp virus (14).

During the past several years, we, along with colleagues in collaborating laboratories, have made and characterized four additional reovirus isolates from several species of finfish. The SBR isolate was obtained from striped bass (*Morone saxatilis*) with numerous external hemorrhagic lesions (16) that were collected from the Chesapeake Bay. These fish were also concomitantly infected with a bacterium of the genus *Moraxella*. Another isolate was obtained by Moore and McMenemy (19) from sex products of different populations of normal Atlantic salmon being routinely sampled in New Brunswick, Canada. A third strain (SRV), characterized by Marshall et al. (18), was isolated from rainbow smelt (*Osmerus mordax*) suffering mortalities in Canada; a picornavirus-like agent was also isolated (19). The fourth isolation was made in Spain from cultured turbot (*Scophthalmus maximus*) that also were infected with a bacterium, a member of the genus *Vibrio* (20). Both agents were recovered from the kidneys and livers of affected turbot in a population exhibiting chronic mortality. The typical syncytial-type CPE produced by these agents is shown in Fig. 1.

Molecular characterization. Samal et al., (21) compared the biochemical characteristics of the above four reoviruses. The genome of each isolate was composed of 11 segments of dsRNA and each isolate had a unique electropherotype in polyacrylamide gels. Agarose gel electrophoresis showed similar RNA profiles for all three North American isolates whereas the European isolate was quite different. Similarly, reciprocal RNA-RNA blot hybridizations, showed cross-reactions among the three North American strains but not between the North American strains and the European (turbot)



(a)



(b)

Fig. 1. Syncytia produced by the turbot aquareovirus. (a) Normal chinook salmon embryo (CHSE-214) cell monolayer (×225); (b) large syncytium in CHSE cells infected with the turbot aquareoviruses (×225).

isolate (22). No genetic relationship was found between any of the four isolates and the mammalian group A rotavirus SA 11 (22). We have compared 19 of the aquareovirus isolates by dot-blot hybridization and have defined 5 genetic groups based on their cross-reactivity or lack thereof. Three of the groups are monotypic containing only one virus while the remaining 2 groups contain the other 16 isolates (Lupiani, Subramanian, Hetrick, and Samal, unpublished information).

Analysis of virion proteins revealed that each virus had five structural proteins ranging in M_r from 130 to 40 Kd. Although each isolate had a unique polypeptide profile, their overall polypeptide patterns were similar (23). All of the other aquatic reoviruses that have been characterized have similar genome and protein profiles except for the grass carp virus which has 10 rather than 11 segments of dsRNA. These results are similar to those reported previously by Winton et al. (24) who characterized five of the first aquareoviruses isolated.

Hsu et al. (12) compared the biochemical characteristics of the landlocked salmon virus (LSV) with four other aquatic reoviruses, chum salmon virus (CSV), catfish reovirus (CRV), golden shiner virus (GSV), and 13p2 virus isolated from the American oyster (*Crassostrea virginica*). A comparison of the electropherotypes of the RNA genomes and virion proteins showed LSV to be closer to CSV in RNA pattern but more like 13p2 in its protein profile. However each of the viruses had characteristic RNA and protein patterns and could be readily distinguished from each other. In an analogous comparison, Varner and Lewis (13) found that the electrophoretic profiles of genomic segments and polypeptides of the angelfish reovirus displayed similar, but distinct, migration patterns from those found with CSV, GSV, and CRV.

The optimal temperature of replication for those viruses studied was in the 15–20 °C range except for the angelfish virus which grew best at temperatures of 22–27 °C.

Pathogenicity. Controlled transmission studies have been attempted to date with only a few of the new reovirus isolates. The general findings, as detailed below, are similar to those reported for the previously studied aquareoviruses in that they exhibited little or no virulence. Intraperitoneal inoculation of rainbow trout with the coho salmon virus did not cause mortalities nor were any external lesions evident (11). The virus did replicate in the fish and focal necrotic lesions were noted in the livers of some animals. The turbot virus produced only low grade mortalities in fry but not in older turbot and had only low virulence for rainbow trout fry. This virus was isolated from turbot that were also infected with a *Vibrio* sp. The *Vibrio* was shown to be pathogenic for rainbow trout, but, treatment of the fish with oxolinic acid was not totally effective in arresting the mortalities which suggests the turbot reovirus may have some role in the pathologic condition (20). It is noteworthy that one year after the initial outbreak, both agents were isolated from surviving turbot that developed the same pathological condition. Like the turbot virus, the striped bass virus was isolated from fish suffering from a disease with a possible mixed etiology. Juvenile striped bass inoculated with the virus did not suffer mortalities but virus was recoverable from the visceral organs for up to 10 weeks postinoculation. The *Moraxella* strain involved in the syndrome was pathogenic for rainbow trout with a mean LD_{50} of 10^5 organisms (16).

Rhabdoviridae

Table 2 lists the major rhabdoviruses described from fish to date. Three new viruses from brown trout, pike, and pike-perch are discussed as are several rhabdoviruses from snakehead, whose characteristics have become better known since 1988.

Snakehead rhabdoviruses. (a) *Isolation.* A rhabdovirus was isolated from striped snakehead (*Ophicephalus striatus*) and freshwater eel (*Fluta alba*) suffering from a severe ulcerative disease in southeast Asia (38). The virus was isolated from different areas of Thailand and Burma and has been named ulcerative disease rhabdovirus (UDRV) because it was thought to be the cause of the ulcerative condition plaguing fish in several Southeast Asian countries. The virus appears to be distinct from previously described rhabdoviruses both in its cell culture spectrum and the fact that it is neutralized only by homologous antiserum and not by antisera to other rhabdoviruses (38). Its role as a pathogen for snakehead fish however, has not been demonstrated, but it is a vesiculovirus type that is serologically distinct from a second lyssavirus type, the snakehead rhabdovirus (SHRV), isolated by Wattanavijarn et al. (34) and from five other fish rhabdoviruses (39).

(b) *Cell culture spectrum.* The UDRV was cytopathic in the commonly used bluegill fry (BF-2), fathead minnow (FHM), and Atlantic salmon (AS) cell lines and in cell lines established from the tissues of fish indigenous to Southeast Asia, including striped snakehead (SSN-1), blotched snakehead (BSN), red snakehead (RSN), and climbing perch (CP) cell lines (38). No cytopathic effects were seen

Table 2. Major rhabdoviruses found in fish

Virus	Naturally infected hosts	Geographic distribution	Source tissue	Disease	Reference
Viral hemorrhagic septicemia virus (VHSV, Egtved virus, Cod ulcus) (Lyssavirus)	Salmonids[a]; pike (*Esox lucius*); Pacific and Atlantic cod (*Gadus macrocephalus*, *G. morhua*); grayling (*Thymallus thymallus*); white fish (*Coregonus* sp.)	Europe, West Coast N. America	Systemic, cutaneous	Yes/no	(25)
Infectious hematopoietic necrosis virus (IHNV) (Lyssavirus)	Salmonids[b]	Western N. America, France, Italy, Germany, Belgium, Luxembourg, Japan, Taiwan, Korea, China	Systemic	Yes	(26)
Hirame rhabdovirus (HRV) (Lyssavirus related to IHNV)	Japanese flounder (*Paralichthys olivaceus*); ayu (*Plecoglossus altivelis*)	Japan	Systemic	Yes	(27)
Spring viremia of carp virus (SVCV) (Vesiculovirus)	Cyprinids[c] sheatfish (*Silurus glanis*)	Europe	Systemic	Yes	(28)
Pike fry rhabdovirus (PFRV) (Vesiculovirus related to SVCV)	Northern pike; grass carp; tench (*Tinca tinca*); white bream (*B. joerkna*); brown trout	Europe, including Russia	Systemic	Yes	(29)
Eel rhabdoviruses (EVA, EVEX) (Lyssavirus)	American and European eels (*Anguilla rostrata*, *A. anguilla*)	Japan and Europe	Systemic	No	(30)
Eel rhabdovirus (B_{12}) (Lyssavirus)	European eels	France	Systemic	No	(31)
Perch/pike perch rhabdovirus (Vesiculovirus)	Perch (*Perca fluviatilis*); Pike perch (*Stizostedion lucioperca*)	France	Systemic	Yes	(32,40)
Ulcerative syndrome and snake head rhabdoviruses (2 types) UDRV – Vesiculovirus SHRV – Lyssavirus	Snakeheads (*Ophicephalus striatus*), (*O. punctatus*); Freshwater eel (*Fluta alba*), (*O. striatus*)	Southeast Asia	Systemic	No	 (33) (34)
Salmo trutta carpione rhabdovirus	Brown trout, *Salmo trutta* (m. *carpio*)	Italy	Systemic	Yes	(Bovo, personal communication)
Brown trout rhabdovirus	Brown trout, *Salmo trutta* (m. *lacustris*)	Finland	Systemic	Yes	(35)
Pike rhabdovirus	Northern pike	Denmark	Systemic	Yes	(P.E. Vestergård Jørgensen, personal communication)
Rhabdovirus salmonis	Rainbow trout	USSR	Systemic	Yes	(36)
Rio Grande cichlid rhabdovirus	Rio Grande perch (*Cichlasoma cyanoguttatum*)	USA	Systemic	Yes	(37)

[a] *Oncorhynchus mykiss*, *O. tshawytscha*, and *O. kisutch*.
[b] *O. mykiss*, *O. tshawytschua*, *O. nerka*, *O. kitsutch*, *O. gorbuscka*, *O. rhodurus*, *O. masou*, *O. keta*, *O. macrostomus*, *S. salar*, and *Salvelinus leucomaenis*.
[c] *Cyprinus carpio*, *H. nobilis*, and *C. carassius*.

in the CHSE-214, RTG, or EPC cell lines and this refractory nature differentiates UDRV from other rhabdoviruses including viral hemorrhagic septicemia (VHS), infectious hematopoietic necrosis (IHN), eel virus American (EVA), European eel virus (EVEX), spring viremia of carp virus (SVCV), pike fry rhabdovirus (PFRV), hirame rhabdovirus (HRV), perch rhabdovirus (PRV), and SHRV which all replicate in one or more of these cell lines. Viral infectivity for the susceptible lines varied from titers of $10^{2.7}$ to $10^{6.9}$ $TCID_{50}$ per ml depending on the cell line employed, but generally the titers were lower than those normally obtained with fish rhabdoviruses. The cytopathology is characterized by the appearance of granular and rounded cells which eventually lyse. In some cultures, initial CPE resulted in only partial destruction of the monolayer after which normal cells grew in and reestablished confluency. Generally, such healed monolayers become carrier cultures of the virus (38). The virus replicated optimally at 24–30 °C in snakehead fin and carp cell lines. Growth curves in epithelioma populosum cyprini (EPC) cells with SHRV showed a latent period of 4 h with all cell-associated virus being released by 14 h.

(c) *Virus characterization.* The SHRV was bacilliform, 60–70 nm in width and 180–200 nm in length, and contained five structural proteins with Mr of > 150, 69, 48, 26, and 20 Kd. The 69 Kd protein was glycosylated. Thus this virus is similar to the salmonid lyssaviruses, IHNV and VHSV (39). However, cross-neutralization tests have shown that UDRV is antigenically distinct from seven other recognized rhabdovirus pathogens of fish, that is VHSV, EVA, IHNV, SVCV, PFRV, EVEX, and PRV (39).

Brown trout rhabdoviruses. (a) *Isolation.* Two rhabdoviruses recently reported from brown trout (*Salmo trutta*) are potentially "new" additions to previously established groups of fish rhabdoviruses (Table 3). Koski et al. (35) reported the isolation of a rhabdovirus from a group of brown trout (*S. trutta lacustris*) from a farm in northern Finland. The fish were suffering a low grade mortality at water temperatures of 16 °C. External and internal signs were similar to those described for other systematic rhabdovirus infections in salmonids. The virus was isolated from internal organs using the BF-2, FHM, and EPC cell lines with CPE occurring in 2–5 days at 15 °C in all three cell lines.

A second rhabdovirus was isolated from brown trout (*S. trutta carpio*) from northern Italy (Bovo et al., personal communication). The virus was associated with mortality among three month-old brown trout reared in river water. The fish showed signs typical of systemic rhabdovirus infections (e.g. VHSV) in salmonids. The virus was isolated using standard cell lines including RTG-2, BF-2, and EPC.

(b) *Virus characteristics.* Both brown trout rhabdoviruses have only been partially characterized. The virus from brown trout in Finland (903/87) was 170 nm by 100 nm in negative stains of partially purified virions. This virus was not neutralized by a panel of polyclonal sera made to each of the major groups of rhabdoviruses, although the B_{12} eel lyssavirus was not included in the comparisons. Similarly, there was little cross-reaction in ELISA tests with the same panel of viruses and antisera. An examination of the polypeptides of the virus 903/87 has not yet been reported.

Bovo et al. (personal communication) found that the polypeptide profile of the caripone virus was similar to VHSV and other lyssaviruses. Ad-

Table 3. Picornavirus-like agents described in finfish

Virus	Naturally infected host	Geographic distribution	Source tissue	Disease	Reference
Rainbow smelt	*Osmerus mordax*	Canada	Viscera	No	(19)
European smelt	*Osmerus eperlanus*	Germany	Fin tumors	?	(44)
Salmonid picorna	*Salmo salar*	Washington, USA	Viscera	No	(45)
	Oncorhynchus mykiss	Washington, USA	Ovarian fluid	No	(47)
	Oncorhynchus clarki	California, USA	Ovarian fluid	No	(46)
	Oncorhynchus mykiss	California, USA	Ovarian fluid	No	(46)
	Salmo trutta	California, USA	Ovarian fluid	No	(46)
	Salvelinus fontinalis	California, USA	Ovarian fluid	No	(46)
Barramundi	*Lates calcarifer*	Australia	Larvae	Yes	(48)
Turbot	*Scophthalmus maximus*	Norway	Larvae	?	(50)
Japanese parrotfish	*Oplegnathus fasciatus*	Japan	Viscera	Yes	(51)
Redspotted grouper	*Epinephelus akaara*	Japan	Larvae, juvenile	Yes	(52)

ditionally, some cross-reactions with polyclonal and monoclonal antibodies to VHSV were detected in immunofluorescence tests. Further studies on the potential relationship of this virus to VHSV are warranted.

Both brown trout and other salmonids need to be tested for their susceptibility to these potentially new viruses.

Pike rhabdovirus. (a) *Isolation.* In June of 1989 a group of healthy pike fry (*Esox lucius*) held in Denmark were examined for virus because of their potential exposure to VHSV from a trout farm. During that examination a virus was isolated (P.E. Vestergård Jørgensen, personal communication). The virus (5533) was isolated four days after inoculation of BF-2 cells at 15 °C.

(b) *Virus characteristics.* Initial characterization of the agent indicates it displays the typical size and morphology of other fish rhabdoviruses. In experimental infection trials the agent was pathogenic for pike fry but not rainbow trout. The agent was found to be clearly different from pike fry rhabdovirus (PFRV) in serological studies using hyperimmune rabbit serum produced to both pike viruses. Additionally, the new pike virus (5533) showed no cross-reactions in indirect fluorescence tests with any of 11 other rhabdoviruses representing all of the major groups. The brown trout virus from Finland, the Rio Grande cichlid virus, and *Rhabdovirus salmonis* were not included in these comparisons.

Pike-perch rhabdovirus. A massive mortality among pike-perch (*Stizostedion lucioperca*) alevins from a hatchery in central France in 1990 was attributed to an infection with a systemic rhabdovirus by Nougayrede et al. (40). The fish were 23 days old and were being held at a water temperature of 17 °C.

(a) *Isolation.* The pike-perch rhabdovirus (PPRV) was isolated from moribund fish by standard virological methods using the RTG-2 and EPC cell lines incubated at 15 °C. After 6–7 days, the EPC cells were completely destroyed, but only small plaques were detected on the RTG-2 line and these spontaneously vanished by 10 days. Later studies showed the virus grew well in the BF-2 line and the WO line from walleye ovary.

(b) *Viral characteristics.* Partially purified virus particles with typical rhabdovirus morphology measured 120–130 nm in length by 70–80 nm in diameter. The virus was not neutralized by antisera prepared to VHSV, IHNV, PFRV, SVCV, and two eel viruses, however, immune trout serum prepared against the perch rhabdovirus neutralized the pike-perch isolates (32). The serological evidence for their similarity and their isolation from the same geographical region (albeit 10 years apart) suggest that the pike-perch and perch rhabdoviruses are related agents. The virulence of the pike-perch virus is now under investigation.

Paramyxoviridae

This family of viruses contains few pathogens for fish. In 1985, Winton et al. (41) described the isolation of a paramyxovirus from stocks of normal adult chinook salmon. The virus has not been demonstrated to be pathogenic for chinook salmon but it has the unique characteristic of being the only fish virus known to hemagglutinate erythrocytes from fish, mammals, and birds. This virus resembles other paramyxoviruses in its ability to persistently infect cell lines (42).

A second paramyxovirus has been reported by Miyazaki et al., (43) to cause epidermal necrosis in black sea bream (*Acanthopagrus schlegeli*) larvae, however, the agent has not been cultivated to date. The disease in sea bream is characterized by necrosis of epithelial cells in the epidermis of the fins and body surface, gills, intestinal and oral mucosa. The paramyxovirus-like virions were spherical enveloped particles approximately 300–370 nm in diameter and were readily seen in the cytoplasm of infected cells.

In natural infections, the disease occurred among 25–30-day-old fish and resulted in 100% mortality within a few days time. The disease was transmissible by immersion of fish in an ultrafiltrate of naturally infected larvae. Diseased fish were observed 4–6 days after immersion and, as with the natural infection, mortalities approached 100%. Experimentally infected fish showed the same pathology as the naturally infected fish.

Picornaviridae

Until recently there have been few reports of picornavirus-like agents occurring in fish. However, during the past few years several of these agents have been isolated from, or seen in, the tissues of diseased fish (Table 3). Moore et al. (19) reported the isolation of a picornavirus from landlocked rainbow smelt in New Brunswick, Canada. The virus was isolated in CHSE-214 cells from fish displaying no clinical signs of disease. The CPE developed as early as four days postinoculation and many cytoplasmic nonenveloped viral particles (20–30 nm) were seen in the cells. Transmission studies with the agent in brook trout did not result in disease but virus was isolated from all 10 fish that were inoculated. Ahne et al. (44) isolated a picornavirus-

like agent from tumor-like lesions on the fins of adult European smelt (*Osmerus eperlanus*) using CHSE-214 cells as the host system. In ultrathin sections of infected cells, icosahedral particles approximately 30 nm in diameter were seen in the cytoplasm by electron microscopy.

A small RNA virus was isolated from Atlantic salmon (*Salmo salar*) that were survivors of an unexplained mortality that swept through their freshwater rearing site in Washington State, USA. (45). The virus grew optimally in CHSE-214 cells at 15 °C producing numerous syncytia. The virions were hexagonal in morphology with a mean diameter of 39.5 nm, nonenveloped, and had an RNA genome. Waterborne exposure and i.p. injection of the virus into Atlantic salmon did not cause mortalities nor was virus recovered postinfection.

Hedrick et al. (46) reported isolation of a small RNA virus from numerous salmonid broodstocks in California. Isolations were made from ovarian fluids of adult cutthroat trout (*O. clarki*), rainbow trout (*O. mykiss*), brown trout and brook trout (*Salvelinus fontinalis*), and from kidney and spleen tissues of juvenile brown and brook trout. However, the virus was not associated with above normal losses in these species. The most sensitive cell line was CHSE-214 and the virus had an optimal growth temperature of 15 °C and a range of 4–20 °C. Transmission studies did not produce mortalities but virus was recovered for periods up to 3–5 weeks following waterborne exposure of rainbow and brown trout but not chinook or coho salmon (46).

Eaton and coworkers (47) reported the isolation of a picornavirus-like agent from the ovarian fluid of steelhead in Washington State, USA. The virus grew in a variety of cell lines and produced a unique type of cytopathology characterized by multinucleated cells which later formed aggregations of cells containing numerous gas-filled vacuoles. The virions were 25–30 nm icosahedrons with a single stranded RNA genome. No deaths occurred among rainbow trout inoculated with the virus although virus was detected in trout tissues for at least four weeks postinjection. This virus may be the same as the one isolated from Atlantic salmon from the same geographic region (45). The size differences reported (25–30 for the steelhead virus and 39.5 nm for the Atlantic salmon virus) may be due to the fact that the steelhead virus dimensions were determined in thin sections of tissues while the Atlantic salmon virus preparation was partially purified and negatively stained.

A major problem associated with the culture of barramundi (*Lates calcarifer*) has been the high mortality rates (50–90%) suffered by larvae under hatchery conditions. Glazebrook et al. (48) described a picornavirus-like agent in degenerative areas of the brain and retina of 2–3 week-old larvae. The virus was 25–30 nm in diameter, replicated solely in the cytoplasm, contained RNA, and produced inclusion bodies. This virus is the first to be identified in a cultured species of native finfish in Australia. Results of transmission studies indicate that the virus is highly infectious and is transmitted from diseased to healthy fish within four days of contact. Attempts to isolate the virus from infected larvae using RTG-2, FHM, BF-2, and the BHT line from barramundi heart were unsuccessful. Some level of control has been achieved by the use of disinfection procedures between production lots, which has reduced the massive mortalities experienced in earlier outbreaks with the virus (49). Further characterization of the agent will have to await development of a susceptible cell culture system.

Bloch et al. (50) reported an outbreak of encephalomyelitis among larval turbot that caused heavy mortalities. Transmission electron microscopy of brain tissue revealed cells containing dense pleomorphic bodies filled with virus-like particles. The particles appeared to be round and averaged 29 nm in diameter. They have not yet been grown in cell culture.

Yoshikoshi and Inoue (51) described a disease called viral nervous necrosis which causes nearly 100% mortality in larval Japanese parrotfish. Lower mortalities occurred in juvenile parrotfish and the observed lesions were less extensive than those seen in the larvae. Viruses, approximately 34 nm in diameter, were seen in the central nervous system of diseased fish but the authors made no attempt to classify them. Again, development of a susceptible cell culture host is needed before more virus characterization can be attempted.

A viral disease has also been observed among hatchery-reared larvae and juvenile redspotted grouper (*Epinephelus akoara*) in Japan (52). The conditions of infected fish were similar to those described above for barramundi, parrotfish, and turbot. However, in contrast to these, the groupers also suffered significant mortalities as juveniles and there was no significant difference in the severity of lesions noted in larval and juvenile stages. Transmission electron microscopy showed lytic degeneration of neurons in the brain and retina and numerous hexagonal virus particles (28 nm in diameter) in the cytoplasm with paracrystalline arrays in membra-

nous structures often being apparent. Attempts to isolate the virus in FHM or EPC cells were not successful.

Retroviridae

Many fish populations have been reported to develop a high incidence of tumors, but until recently retrovirus-like particles had only been described in three of these; a dermal sarcoma of walleye, *Stozostedion vitreum vitreum* (53), lymphosarcoma of northern pike, *Esox lucius* (54), and swimbladder fibrosarcoma in Atlantic salmon (55). Walleye develop a dermal sarcoma that can involve 27% of adults in the spring although the tumors regress in the summer (56,57). The tumor appears histologically malignant but it neither invades nor metastasizes (58). The tumor is readily transmitted by inoculation of cell-free filtrates into susceptible fish (59) and water temperature affects both the incidence and time required for tumor development (60). Preliminary hybridization studies indicated that a linear unintegrated species was the major form of viral DNA in the tumors (61). Martineau et al. (62) concentrated retrovirus particles from a homogenate of 20 pooled tumors and the fraction containing the particles had a density of 1.18 g/ml and high reverse transcriptase activity. The complete viral DNA has been cloned directly from tumor DNA (62). Viral DNA was readily detected by southern blots in all six tumors analyzed and from a comparison with copy number standards, it was estimated that there were from 7 to more than 50 viral copies per tumor cell.

Another putative retroviral disease is a plasmacytoid leukemia (PL) in chinook salmon (63) which has been diagnosed at three different netpen farms in British Columbia since 1987. Mortality was 80% at one site. The disease has been experimentally transmitted to coho salmon, Atlantic salmon, rainbow trout, and sockeye salmon using kidney tissue from an infected chinook salmon as inoculum. Reverse transcriptase activity associated with fractions from sucrose gradients made from infected tissues provided preliminary evidence for the viral etiology of PL. Attempts are underway to establish a tissue culture system to facilitate further characterization of this agent.

Lentivirus particles have been seen by electron microscopy in thin sections of fibromas or fibrosarcomas occurring in hooknose (*Agonus cataphractus*) collected from the Wadden Sea (64). The virus particles were seen in cytoplasmic vacuoles of the tumor cells.

The spontaneous release of typical C-type retrovirus particles from four cell lines initiated from three warmwater fish species was reported (65), two from striped snakehead (*Ophicephalus striatus*), one from a climbing perch (*Anabas testudineus*), and one from snakeskin gourami (*Trichogaster pectoralis*). Virus pelleted from cell culture fluids banded at 1.16 g/ml in sucrose gradients and a peak of reverse transcriptase activity and numerous 85–90 nm particle numbers were present in this fraction. Interestingly, all four isolates produced CPE in a bluegill fry (BF-2) cell line (64). Their relationship to tumors in fish is unknown.

The association of retroviruses with tumors in fish will be covered in Bowser's more in-depth review later in this issue.

Coronaviridae

A coronavirus was isolated from laboratory-held common carp dying from an acute infection whose external signs consisted only of erythematous skin on the abdomen (66). The virus was isolated in fathead minnow (FHM) cells from the kidney, liver, and spleen of moribund fish. Other cell lines were also susceptible including BF-2, EPC, and RTG-2 but the highest yield was obtained from the FHM line with the optimal temperature being 20 °C. The isolate was classified as a coronavirus because of its following characteristics: enveloped virions 60–100 nm in diameter, buoyant density of 1.21 g/ml, inactivation at pH 3.0, heat lability (56 °C), resistance to IUdR. The disease could be transmitted by immersing carp fry in 20 °C water containing the virus. Affected fish had abdominal extension and a hemorrhagic abdomen. Histopathology revealed hepatic and renal necrosis as well as necrosis in the intestinal tunica mucosa (66).

DNA VIRUSES

Herpesviridae

Members of the family *Herpesviridae* are well represented in fish (Tables 4 and 5) with 17 seemingly distinct agents now having been isolated from or observed in fish (67). Recently, serious epizootics in both lake trout, *Salvelinus namaycush*, and white sturgeon, *Acipenser transmontanus*, have been found to be caused by newly recognized herpesviruses. Reports by both Bradley et al. (88) and McAllister and Herman (89) described massive mortalities among lake trout reared in the Great Lakes region of the USA. Although epitheliocystis was suspected in the initial outbreaks (88,89), the underlying cause was found to be a herpesvirus clearly

Table 4. Major herpesviruses isolated from fish

Virus	Naturally infected hosts	Geographic distribution	Source tissue	Disease	Reference
Salmonid Herpesviruses					
Type I *Herpesvirus salmonis*	Rainbow trout (*Oncorhynchus mykiss*)	West Coast N. America	Ovarian fluid	Experimental (Exp.)	(68,98)
	Steelhead trout (*O. mykiss*)	West Coast	Ovarian fluid	No	(69,98)
Type II (examples of isolates)					
NeVTA	Kokanee salmon (*O. nerka*)	Japan	Fry	Yes	(70)
OMV	Masou salmon (*O. masou*)	Japan	Ovarian fluid	Exp.	(71,72)
YTV	Yamame salmon (*O. masou*)	Japan	Tumor	Exp.	(73)
CSTV	Coho salmon (*O. kisutch*)	Japan	Tumor	Exp.	(74)
Ictalurid Herpesvirus					
Channel Catfish Virus (CCV)	Channel Catfish (*Ictalurus punctatus*)	USA, Central America	Fry/juveniles	Yes	(75)
Cyprinid Herpesvirus					
Herpesvirus cyprini	Fancy carp (*Cyprinus carpio*)	Japan	Epidermis	Exp.	(76)
Percid Herpesvirus					
Herpesvirus vitreum	Walleye (*Stizostedion vitreum*)	Canada	Epidermis	No	(77)
Anquillid Herpesvirus					
Herpesvirus anguillae	European eel (*Anguilla anguilla*)	Japan	Viscera	Yes	(78)
	Japanese eel (*A. japonica*)	Japan, Taiwan	Viscera	Yes	(78,93)
Acipenserid Herpesvirus					
White sturgeon herpesvirus	White sturgeon				
Type I	(*Acipenser transmontanus*)	West Coast N. America	Integument	Yes	(79)
Type II			Integument/ovary	Yes	

Table 5. Major herpesviruses identified by electron microscopy

Virus	Naturally infected hosts	Geographic distribution	Source tissue	Disease	Reference
Carp pox (*Herpesvirus cyprini*)	Common carp (*Cyprinus carpio*)	Europe	Epidermis	No	(80)
Golden ide virus (*Herpesvirus cyprini*)	Golden ide (*Leuciscus idus*)	N. America	Epidermis	No	(81)
Pacific cod herpesvirus	Pacific cod (*Gadus macrocephalus*)	West Coast	Epidermis	No	(82)
Pike epidermal proliferative herpesvirus	Northern pike (*Esox lucius*)	Canada	Epidermis	No	(83)
Herpesvirus scophthalmi	Turbot (*Scophthalmus maximus*)	U.K.	Epidermis/gills	Yes	(84)
Sheatfish herpesvirus	Sheatfish (*Silurus glanis*)	Central Europe	Epidermis	No	(85)
Smooth dogfish herpesvirus	Smooth dogfish (*Mustelus canis*)	N. America	Epidermis	Yes	(86)
Smelt papillomatous virus	Smelt (*Osmerus eperlanus*)	Europe	Epidermis	No	(87)
Salmonid herpesvirus Type III					
Viral epizootic epitheliotropic virus	Lake trout (*Salvelinus namaycush*)	N. America	Epidermis	Yes	(88,90) (89,91)
Atlantic salmon papillomatosis	Atlantic salmon (*Salmo salar*)	Finland, USSR	Epidermis	Yes	(97)
Angelfish herpesvirus	Angelfish (*Pterophyllum altum*)	Europe	Spleen	Yes	(92)
Japanese flounder virus	Japanese flounder (*Paralichthys olivaceus*)	Japan	Epidermis	Yes	(94)

distinct from the previously described salmonid herpesviruses type 1 or 2 (90,91). Additional herpesviruses were identified as the cause of mortality in angelfish (*Pterophyllum altum*) (92), Japanese eels (*Anguilla japonica*), and European eels (*A. anguilla*) by Sano et al. (78). A similar or identical eel herpesvirus has recently been described in Japanese eels in Taiwan by Ueno et al. (93). Herpesviruses associated with epidermal hyperplasia and necrosis were identified from larval and juvenile Japanese flounder (*Paralichthys olivaceus*) (94) and the white sturgeon (79). None of these newly described herpesviruses appear to be related and they therefore are discussed individually.

Epizootic epitheliotropic disease virus (EEDV). Epizootic mortalities among hatchery-reared juvenile and yearling lake trout in the Great Lakes region were investigated by Bradley et al. (88,90) and McAllister and Herman (89,91). Although Bradley et al. (88) and McAllister and Herman (89) initially indicated that the chlamydial agent causing epitheliocystis might have been responsible, both groups now agree that an epitheliotropic herpesvirus is the underlying factor in mortality. Affected fish are lethargic and show episodes of erratic swimming. Hemorrhages may be seen in the eye, or occasionally the mouth, but the more common signs are a mucoid mottled appearance of the skin with secondary fungal invasion (91). Mortalities may approach 99% in affected populations (90). The virus has not been grown in cell culture although it has been observed in infected epidermal cells and concentrated by gradient centrifugation from tissue (90). The virions resemble typical herpesviruses with a capsid of 100–105 nm surrounded by an envelope of 220–230 nm (90). Virions with a tailed appearance were detected in addition to hexagonal particles (91). These tailed particles have been found with several epitheliotropic herpesviruses from fish (79,93).

The natural and experimental host range of EEDV appears to be only lake trout. Brown trout, Atlantic salmon, brook trout, and rainbow trout were not susceptible to experimental infections with EEDV (91). Histopathological signs of infection are present in the skin, being most pronounced in the oropharyngeal region early in infection, but can be found throughout all areas of the integument later in infection. Epidermal or Malpighian cells are swollen, contain an acidophilic granular cytoplasm with enlarged nuclei which may contain an acidophilic inclusion.

The virus can be transmitted to lake trout by exposure to both filtered homogenates or moribund fish at water temperatures of 10–12 °C. Consistent

transmission occurred with 0.45 μm filtered extracts from affected skin, but Bradley et al. (90) failed to get infectivity after filtration through a 0.22 μm membrane. Mortality is severe with up to 100% of the infected fish dying by 15 days postimmersion challenge. The virus has yet to be isolated in cell cultures derived from salmonid fish including primary lines derived from lake trout (P.E. McAllister, personal communication).

White sturgeon herpesvirus (WSHV). There is now evidence for two epitheliotropic herpesviruses (WSHV type 1 and 2) from hatchery-reared juvenile white sturgeon (79). The viruses have been repeatedly isolated from juvenile sturgeon suffering low grade to moderate mortalities during the early rearing phases. The herpesviruses (and the iridoviruses to be discussed later) are believed to be major contributors to juvenile mortality commonly encountered in white sturgeon culture.

(a) *WSHV-1.* This virus was the first herpesvirus to be isolated from white sturgeon and was associated with epidermal hyperplasia followed by necrosis (79). Mortalities due to WSHV-1 were seldom greater than 20% during any one episode. The virion is typical of other herpesviruses with a hexagonal capsid (mean diameter of 110 nm) containing a deeply staining core surrounded by the tegument and an external envelope 230 nm in diameter. White sturgeon are currently the only known host for WSHV-1. The integument, particularly around the oropharyngeal mucosa, is the main target of the virus and acanthosis and necrosis of the affected epithelium is evident. The virus was transmitted to, and caused mortality among, juvenile white sturgeon but not lake trout following exposure to bath challenges with virus from cell culture.

The WSHV-1 can be isolated from extracts of skin using a recently established cell line from the epidermis of white sturgeon (WSSK-1). There was no evidence for virus replication in two other lines from white sturgeon heart (WSH-1) and spleen (WSS-2) or in the CHSE-214 and EPC lines derived from chinook salmon and common carp, respectively. Cytopathic effects in WSSK-1 cells were characterized by syncytia 2–4 days after inoculation of cells at 15 °C. The virus grew in WSSK-1 cells at 10 °C, 15 °C, and 20 °C but not at 5 °C or 25 °C.

The association of WSHV-1 with mortality among farm-reared and experimentally infected fish indicates that the virus can be one cause of mortality often observed in the early rearing phases of juvenile white sturgeon.

(b) *WSHV-2.* In 1990, a second herpesvirus (WSHV-2) was isolated from internal organs of captive adult white sturgeon broodstock and later from juvenile mortalities occurring on commercial farms (Watson, Groff, and Hetrick, in preparation). The biological properties of this second herpesvirus suggest that it differs substantially from WSHV-1 and should be considered a separate and unique agent. The WSHV-2 appears to be both more pathogenic (inducing higher mortality in experimental studies) and can be found both in skin and internal organs of infected white sturgeon. In addition, the virus is more rapidly lytic in cell culture and shows a broader host cell range, replicating in several cell lines from white sturgeon other than the WSSK-1 line. Hyperimmune serum to WSHV-1 did react with WSHV-2 in neutralization and indirect fluorescent antibody tests, and initial investigation of their biochemical properties has shown differences between WSHV-1 and WSHV-2 which in part may explain their unique biological characteristics.

Japanese flounder herpesvirus. Epidermal necrosis among larval and juvenile Japanese or olive flounder was reported in 1985 from Hiroshima Prefecture (95) and in 1986 from Mie Prefecture Japan (96). In both reports, dying fish suffered an epidermal hyperplasia and necrosis syndrome with mortalities to 90%. Typical herpesvirions were observed in affected Malpighian cells with a capsid diameter of 100–140 nm surrounded by an envelope of 190–230 nm (94). The virus could not be isolated in five commonly used fish cell lines but 0.45 μm filtrates made from infected fish readily infected previously healthy flounder following immersion exposures (95). Up to 50% of the virus-exposed fish died during the 14 day experiment and all survivors showed some form of epidermal hyperplasia. Additional filtration studies showed that the virus could pass filters as small as 0.22 μm but not 0.1 μm. In similar filtration studies, Miyazaki et al. (95) found that experimentally exposed flounder experienced a 96% mortality over 9 days with the first deaths beginning 2 days after immersion exposure at 20 °C. Naturally and experimentally infected fish show a cloudy to white external appearance due to the enlarged and hyperplastic epidermal cells. These cells undergo necrosis and may be seen as loosely attached aggregates on the surface of the fish. Affected cells have a vacuolated to granular acidophilic cytoplasm with swollen nuclei which may contain an acidophilic inclusion (95). Internal histopathology was less common although hepatic atrophy and some renal tubular cell epithelial changes were observed. The conclusions by both Iida et al. (96) and Miyazaki et al. (95) were that the epidermis is the major

target and that viral epidermal hyperplasia is a proper name for the disease. The virus has not been grown in cell culture.

Anguillid herpesvirus. A herpesvirus was isolated by Sano et al. (78) from both Japanese and European eels using a cell line developed from eel kidney. This virus was isolated from eels held in an intensive culture system in Japan. The virus has been designated *Herpesvirus anguillae*. The virions are typical of the *Herpesviridae* with respect to the diameters of the capsid and the surrounding envelope. The buoyant density of the virions in $CsCl_2$ was 1.24 g/cm^3 and they contained at least 25 structural polypeptides. The only known hosts for the anguillid herpesvirus are Japanese and European eels. Details on experimental transmission have not been reported. The viruses induce CPE including syncytia and intranuclear inclusion formation at optimal temperatures of 20–25 °C but only on cell lines derived from eel. A one step growth curve is completed in 21 h in eel kidney cells at 25 °C. The eel virus is not related to herpesviruses isolated from channel catfish, carp, or salmonids and as found with other herpesviruses, is most likely quite host specific.

A herpesvirus, with characteristics similar to *H. anguillae*, has been isolated from Japanese eels in Taiwan (93). The virus replicated in several cell lines over the temperature range of 12–33 °C. The virus caused no mortality but was reisolated following experimental exposures of eels and carp.

Atlantic salmon papillomatosis herpesvirus. Serious outbreaks of papillomatous growths among Atlantic salmon in Murmansk province were associated with the observation of herpesvirus-like particles in affected tissue (97). Affected fish experienced a 1–2% mortality per day during October at water temperatures of 6 °C. Affected fish had papillomas appearing as semispherical raised plaques of bluish grey color. The papillomas eventually were sloughed but in some cases these areas became infected with secondary invaders and would then ulcerate. Herpesvirus-like particles were observed in nearly all papillomatous tissues examined. The enveloped particles found in cytoplasmic vacuoles ranged from 200–250 nm. Unenveloped nucleocapsids of 110 nm were found within the nucleus of infected cells. The Atlantic salmon herpesvirus (ASHV) appears to be yet another example of the epitheliotropic herpesviruses now described from fish (Tables 4 and 5). Whether the virus can cause a severe systemic disease among younger Atlantic salmon is yet unknown, but if the causative agent can be isolated in cell culture this hypothesis can be tested. Unfortunately, attempts to isolate ASHV on several salmonid lines have so far been unsuccessful.

Angelfish herpesvirus. Herpesvirus-like particles were observed in adult angelfish (*Pterophyllum altum*) imported to Denmark from the Amazon (92). Affected fish were from an aquarium and following some changes in the decoration they became ill showing loss of equilibrium and some spiral swimming prior to death. Grossly, moribund fish had hemorrhagic skin, pale gills, and enlarged liver and spleen. No other pathogens were detected.

Virus particles were found in spleen macrophages and monocytes within the stroma and ellipsoids. The virions found in the nucleus lacked an envelope and measured 100 nm in diameter. Enveloped virions within the cytoplasmic vacuoles were 135 nm or larger with irregular shapes. The virus was found principally in the spleen where dilation of the sinusoids was accompanied by necrosis of the stromal cells. Hemosiderin accumulations were found in macrophages of the reticuloendothelial system suggesting that a hemolytic anemia was in progress but hematocrits were not reported.

The angelfish, *P. altum*, is the only known host and *P. scalare* sharing the same aquarium with diseased fish were not affected. The route of transmission is unknown but the investigators concluded that infected fish may have carried a latent virus which was activated by an episode of stress brought on by a change in the aquarium decoration. This virus has not been isolated in cell culture.

Iridoviridae

The family Iridoviridae contains viruses found both among invertebrate (principally insects) and poikilothermic vertebrate hosts including fish (Table 6). These viruses were originally grouped together based on their icosahedral symmetry, large double-stranded DNA genome, and the presence of noncellular membrane-derived envelopes (111). There are currently four genera within the family (112). Members of the *Iridovirus* and *Chloriridovirus* are found among insects, while viruses belonging to the *Ranavirus* and *Lymphocystivirus* are known from amphibians and fish, respectively. A genus with goldfish viruses, separate from the four genera, has also been proposed (113). Numerous ranaviruses have been isolated from amphibians, principally from normal adult leopard frogs (*Rana pipiens*) (114). The viruses grouped in this genus show a high degree of DNA homology and have been shown to be pathogenic to tadpoles and certain toads (113). The few comparisons made between amphibian and piscine iridoviruses have

Table 6. Major iridoviruses found in fish

Virus	Naturally infected hosts	Geographic distribution	Source tissue	Disease	Reference
		Viruses isolated in cell culture			
Lymphocystis[a]	Numerous marine and freshwater fishes	Worldwide	Epidermis	Yes	(99)
Carp gill necrosis	Common carp (*Cyprinus carpio*)	USSR	Gill	No	(100)
Cod ulcus virus	Atlantic cod (*Gadus morhua*)	Europe	Epidermis	No	(101)
Epizootic hematopoietic necrosis viruses (EHNV)—isolates all similar to frog virus 3					
Red fin perch	Red fin perch (*Perca fluviatilis*)	Australia	Systemic	Yes	(102)
Sheatfish	Sheatfish (*Siluris glanis*)	Europe	Systemic	Yes	(103)
Catfish	Catfish (*Ictalurus melas*)	Europe	Systemic	Yes	(104)
Eel iridovirus	Japanese eel (*Anguilla japonica*)	Japan	Systemic	Yes	(105)
White sturgeon iridovirus	White sturgeon (*Acipenser transmontanus*)	N. America	Integument	Yes	(115)
Goldfish viruses	Goldfish (*Carassius auratus*)	N. America	Systemic	No	(106)
		Viruses observed by electron microscopy			
Erythrocytic necrosis virus	Numerous marine anadromous fishes	Worldwide	Erythrocytes	Yes	(108)
Chromide cichlid virus	Orange chromide cichlid (*Ectropus maculatus*)	N. America Southeast Asia	Systemic	Yes	(109)
Turbot iridovirus	Turbot (*Scophthalmus maximus*)	Europe	Systemic	Yes	(110)
Red sea bream virus	*Pagrus major*	Japan	Systemic	Yes	(125)

[a]Only freshwater viruses have been isolated.

indicated little to no homology and this has led to their current separation into distinct genera (113).

The role of iridoviruses or iridovirus-like agents as causes of epidermal (lymphocystiviruses) and blood cell disorders (erythrocytic necrosis viruses) in fish are well known. Only in the past six years have iridovirus-like agents been recognized as causes of serious systemic diseases among feral, cultured, and ornamental fish populations (102–104,107,109). Few properties of these systemic agents have been described, but their morphological and biological characteristics clearly separate them from the piscine iridoviruses or iridovirus-like agents associated with lymphocystis, erythrocytic necrosis, cod ulcus syndrome, eel and cyprinid infections, or the recently described epidermal infections in sturgeon (107,115).

Epizootic hematopoietic necrosis virus (EHNV). Epizootic hematopoietic necrosis (EHN) was first detected in feral populations of redfin perch (*Perca fluviatilis*) held in lake Nillahcootie, Victoria, Australia (116). The virus was found to be the cause of large scale mortalities when redfin perch were impounded in net pens. Since the initial reports, the virus has been detected in rainbow trout (117) and has been shown experimentally to infect at least seven species of freshwater fish (118).

The virions of EHNV are composed of a capsid with icosahedral symmetry 150–170 nm in diameter with a dense nucleoid and surrounding envelope (102). The virus is partially inactivated by ether and completely inactivated by treatment at pH 4 or 12 for 1 h or 200 ppm hypochlorite for 2 h. Heating at 60 °C for 15 min also destroys all infectivity. The virus is quite stable in water with no loss in infectivity over 97 days at 15 °C (116). Eaton et al. (119) and Hyatt et al. (120) demonstrated the structural and antigenic properties of the EHNV strains from redfin perch and rainbow trout to be identical. The virus contains a large double-stranded DNA genome, which like the *Ranavirus* FV 3, is highly methylated at cytosine residues (121).

Most major organs of diseased fish were affected but the severe necrosis in the renal and splenic hematopoietic tissues characterized the disease in the two natural hosts infected by EHNV, redfin perch and rainbow trout (116,117). The experimental host range for EHNV, however, includes at least seven species of teleost fish (118).

The hematopoietic tissues of the kidney and spleen are the major targets for EHNV. Focal to diffuse necrosis occurring in the absence of a marked host inflammatory response is evident in renal and splenic hematopoietic tissues. The liver, pancreas, and meninges may also be involved; hepatocytes occasionally demonstrate cytoplasmic vacuoles or basophilic inclusions. The meningeal blood vessels may show congestion and thrombosis. More chronic lesions may also be detected in adults surviving infections.

The virus can be transmitted to natural and experimental hosts by either direct immersion or intraperitoneal injection. Death in susceptible natural hosts typically occurs within four days postimmersion. The virus can replicate at 15 °C in several fish cell lines including RTG-2, FHM, and BF-2. Virus can be identified by IFAT and ELISA tests directly in fish tissues (117) and in infected cell cultures (119,120). Difficulty in producing hyperimmune neutralizing sera has precluded development of a serum neutralization test.

Sheatfish iridovirus. In 1988, complete mortalities were observed among sheatfish (*Silurus glanis*) reared in an intensive recirculation system in Germany (103). Affected sheatfish fry were found to harbor an iridovirus-like agent that was isolated and later characterized by Ahne and co-workers (103). The virus, as found in infected fish tissues and in fish cell lines, has icosahedral symmetry with diameters from 125–135 nm and exists only in the cytoplasm (103). The virus is sensitive to chloroform treatment and is inhibited by IUdR indicating that the virus contained essential lipids in the envelope and a DNA genome.

The virus is extremely pathogenic for sheatfish fry with mortalities of 100% recorded from natural and experimental exposures. Fish begin dying three days after exposure and by eight days all fish are dead. The only known host for the virus is sheatfish. Carp and European eels in the same facility with infected sheatfish were not affected by the virus. The experimental host range of the virus has not been reported. The infection caused by the sheatfish virus is systemic and involves primarily the hematopoietic tissues where concentrations of the virus reach $10^{8.2}$ $TCID_{50}$/g. External signs of infected fish include petechial hemorrhages in the skin and occasionally the eyes and barbels (122). The spleen and kidney are major target organs where necrosis of hematopoietic cells is evident. In-depth studies on the pathological changes following experimental challenges demonstrated a systemic disease with microscopic similarities to that of EHNV in redfin perch (116).

The sheatfish iridovirus virus has been transmitted to fry by immersion or cohabitation. By both routes mortalities can approach 100% over a two week period (123). The virus can be isolated from

infected sheatfish using several fish cell lines including those reported for EHNV. The BF-2 cells incubated at temperatures from 20–30 °C are commonly used and CPE is characterized by cell rounding, presence of cytoplasmic inclusions, and eventual lysis. This CPE begins within 36 h and complete destruction of the cell monolayer occurs by 4–5 days postinoculation.

Catfish iridovirus. An iridovirus-like agent has recently been isolated from a pond population of yellow bullhead (*Ictalurus melas*) in France undergoing an acute hemorrhagic syndrome (104). The entire population succumbed to the infection during a one week episode. Affected fish were transported to the laboratory where virus isolations on the EPC line indicated the involvement of a viral agent.

The virus shares an identical shape to EHNV and sheatfish virus with virions of 150–160 nm. Virions are found only in the cytoplasm of infected cells where they can occur in crystalline arrays. Virus infectivity is reduced by treatments with either chloroform or IUdR (104).

As determined by the complete mortality of the pond population during a short period, the virus is considered to be extremely pathogenic. This is the first known natural virus infection of *I. melas*, which is the only species known to be infected with the virus. Similarities with the virus from sheatfish and redfin perch (121) however, suggest that all of these agents have host ranges greater than the species from which they were isolated.

External signs of infection included those typical for viruses having a tropism for endothelial tissues. These included hemorrhages evident in the pectoral and pelvic girdles, the fins, and internally in the viscera and walls of the abdominal cavity. Microscopic signs of disease included degenerative changes such as acute necrosis of the hematopoietic tissues of the kidney and spleen. Edema and dilation of sinusoids in the liver were also observed.

The virus was transmitted by either intramuscular (im) injection or by cohabitation with experimentally infected fish. Mortality among juvenile bullheads occurred 8 days after intramuscular injections and at 16 days among fish exposed to infected fish at water temperatures of 22–25 °C (104).

(a) *Relationships between amphibian and piscine iridoviruses.* The piscine and amphibian viruses share similarities with respect to broad host cell ranges as demonstrated by overlapping susceptibilities for both FV-3 and EHNV (116,117,121). EHNV also has been shown to infect seven species of teleost fish following natural or experimental routes of exposure (117). The few comparisons of amphibian and fish iridoviruses (113,124) have led to the conclusion that FLDV or lymphocystis virus (127) and GFV, the goldfish iridovirus (106), are clearly separable from the amphibian viruses which compose a more homogeneous group. Eaton et al. (119) were the first to suggest that EHNV had similarities to FV 3, and Hedrick et al. (121) demonstrated, in direct comparisons of the structural polypeptides and cross-reactions with hyperimmune rabbit serum, that FV 3 and EHNV were indeed related to each other and to the two iridoviruses isolated from sheatfish in Germany and catfish in France. These studies indicate that serious fish pathogens may also belong in the genus *Ranavirus*, previously thought to be composed solely of amphibian viruses.

Chromide cichlid virus. An iridovirus-like agent has been identified among orange chromide cichlids (*Etropus maculatus*) imported from Malaysia (109). The virus, observed by electron microscopy, was considered as the cause of a systemic disease resulting in anemia and eventually death of infected fish. Similar viral infections have also been detected among several additional species of cichlids imported from southeast Asia (J.M. Groff, personal communication). The virions of the chromide cichlid virus have icosahedral symmetry and are found in the cytoplasm. Capsids have a diameter of 180–200 nm, as measured directly from cells in infected tissues. Virions contain an electron-dense central core typical of iridoviruses from systemic infections in fish.

Chromide cichlids, as well as other cichlids, can be infected (J. Groff, personal communication). Infected fish are anemic, lethargic, and die without exhibiting many external signs. Internal signs are not marked with pale visceral organs and some hyperemia of serosal vessels is detected. In contrast, the microscopic pathology is striking with the presence of numerous hypertrophic cells arising from the hematopoietic tissues. These cells enlarge, become progressively more amphophilic, and may display crystalline inclusion bodies in the nucleus. These cells are distributed by the circulation and may be found in many locations outside of the hematopoietic tissues.

No experimental transmission studies have been reported, but spread of the disease to new inter- and intraspecific populations of cichlids following introduction of diseased fish in wholesale aquaria suggests the virus can be easily transmitted via the water route (J.M. Groff, personal communication). None of the iridovirus-like agents from cichlids have been isolated using established cell cultures. Further investigations into their relationships to each other

and to other systemic iridoviruses from fish and amphibians will be aided by their isolation.

Red sea bream iridovirus. An epizootic among red sea bream (*Pagrus major*) in Japan was found to be associated with a systemic iridovirus infection (125). Anemia was prominent and the principal cell type infected was presumed to be of hematopoietic origin. Mortality of up to 60% was observed in the cultured population. The virus had a hexagonal shape with a diameter of 200–240 nm. Cytopathic effects were observed in five cell lines tested. The disease was experimentally induced among sea bream fingerlings injected with cell-free homogenates prepared from the spleen of infected fish. The descriptions of the disease resemble that described by Armstrong and Ferguson (109) in chromide cichlids and in other cichlids (J.M. Groff, personal communication) and these may indeed be related strains of the same virus.

White sturgeon iridovirus. Yearly losses among juvenile white sturgeon reared in commercial farms in the states of California (107), and now from Oregon and Idaho, USA, have been associated with an iridovirus-like (WSIV) infection of the integument and gills. The disease due to WSIV was first detected in 1988 as the cause of a catastrophic loss (95%) of juveniles at a small commercial farm (107). The iridovirus (WSIV) associated with this disease was isolated using a newly established cell line (WSS-2) from the host species (126). The virus is relatively large and structurally complex. The inner shell is 180 nm in diameter and contains an electron dense bar of 150 nm. The inner shell is surrounded by an outer capsid 275 nm in diameter. The inner and outer shells are enveloped (107).

Natural infections can result in up to 95% mortality and experimental infections with mortality (80% in 50 days) result from bath exposures of white sturgeon (6.4 g) to virus from infected WSS-2 cells (115). Experimental exposures of chinook salmon, channel catfish, and striped bass indicated their resistance to infection, but lake sturgeon suffered a mild form of the disease (115). The virus has an affinity for the skin and gills and infected cells become enlarged and eventually detach from the epithelium. In heavy infections, large sections of the skin and gill epithelium become disrupted and secondary infections with bacteria and protozoa follow. Cells infected with the virus become hypertrophied and exhibit amphophilic to basophilic staining patterns. Rod-like to globular crystalline structures are often detected in the cytoplasm of these infected cells.

The virus has been found in all farms where adequate numbers of fish have been examined in California and among white sturgeon from facilities on the Columbia River, Oregon, and recently in Idaho on the Snake River. The disease is most severe in sturgeon during the first year and infections have been observed in fish from 3 to 16 inches in length. Survivors of initial infections are suspected to be carriers both in culture facilities and wild populations.

Three continuous cell lines from white sturgeon have been developed and the line from spleen (WSS-2) has been found to support replication of WSIV (126). Virus replication occurs at temperatures between 10–20 °C with an optimum at 15 °C. Cytopathic effects begin 7–10 days after inoculation of WSS-2 cells but may take as long as 21 days. Infected cells greatly enlarge and become progressively more rounded and more refractile than surrounding cells and eventually detach from the substrate.

The WSIV appears to be a newly recognized agent unique to white sturgeon. Although the virus infects skin cells, the cell type involved is the Malpighian cell and not fibroblasts as reported for lymphocystis virus (127). The virus replicates only in cell lines derived from sturgeon. Polyclonal antisera and monoclonal antibodies prepared to WSIV do not react with any of the systemic iridoviruses tested.

The WSIV is considered the most significant viral disease of cultured white sturgeon and its appearance in new geographical regions has increased concern over its impact on wild and captive sturgeon.

SUMMARY

In 1981, Wolf (128) wrote a review on the viral diseases of fish and at that time there were 16 agents that had been isolated in cell culture and 11 others that had been seen by electron microscopy. In his book published in 1988 (1), the numbers had grown to 34 isolated viruses and another 25 that were visualized but not yet isolated. As described here during the past 5 years another 35 new agents have been described in the literature including 9 aquareoviruses, 8 picornaviruses, 6 iridoviruses, 5 herpesviruses, 3 rhabdoviruses, 2 retroviruses, 1 paramyxovirus, and 1 coronavirus. Undoubtedly some of these isolates are similar, if not identical, to previously reported viruses. In this regard, we enter a plea to investigators describing new virus isolates that they carefully compare, or have compared, the relatedness of their isolates with similar agents already known before adding another virus name to the literature. Similarly, we ask that those reviewing manuscripts describing new viral agents require that the authors demonstrate through direct compari-

sons the unique nature of an agent before approving the publication. In this manner, all of us can contribute to an orderly and systematic grouping of the many viruses found in fish.

We can anticipate that this number will steadily increase as both the number of trained personnel and the number of species under cultivation increases.

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