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ORIGINAL ARTICLE

Histopathological evaluation and molecular detection of natural *Iridovirus* infection in cultured grouper fish in Malaysia

M . Hazeri[1] · M. D. Hassan[2] · Y. Abba[3] · A. R. Omar[3] · Z. N. Allaudin[3] · M. Soltani[4] · R. H. Hamdan[2] · N. F. A. Mohamad[2] · S. M. Raina[2] · M. S. Vishkaei[5]

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Abstract *Iridovirus* is the causative agent of grouper iridovirus infection (GIV), which causes severe epizootics resulting in large-scale mortalities and huge economic losses in cultured and marine fishes worldwide. The current study evaluated the gross and histopathological lesions, and molecular detection of GIV in naturally infected grouper farms in Malaysia. A total of 150 moribund fish showing different clinical signs, presented to the Aquatic unit were used for this study. The fish were necropsied and visceral organs (spleen, kidney, heart, liver, intestine, brain, eyes, gills, and some parts of skin with ulcer) were collected and fixed in 10 % buffered formalin for histopathological processing and evaluation. Molecular detection of the virus was done by polymerase chain reaction (PCR). Grossly, the groupers had ulceration on the operculum and close to the caudal fin with hemorrhages on the margin of the caudal fin. In some fish, there was necrosis of the caudal fin, sloughing of the epidermis, dermal ulceration and popeyes Histological sections showed large basophilic cytoplasmic inclusions and vacuolations in the cytoplasm of hepatocytes. Basophilic or eosinophilic enlarged cells with the presence of mononuclear cellular infiltrations were seen in the kidney, liver, eye and gills, which is the distinctive feature of this disease. PCR detection showed positive amplification from 27 groupers. Based on the gross, histopathological and molecular detection GIV infection was established.

Keywords Grouper · Histopathology · PCR · Iridovirus · GIV

✉ M. D. Hassan
hassanmd@upm.edu.my

✉ Y. Abba
yabbavet@gmail.com

[1] Institut Biosains, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

[2] Department of Veterinary Clinical Studies Faculty of Veterinary Medicine, Universiti Putra Malaysia, UPM, 43400 Serdang, Malaysia

[3] Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

[4] Department of Aquatic Animal Health, University of Tehran, Tehran, Iran

[5] Department of Food Biotechnology, Faculty Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

Introduction

Iridoviruses have been isolated for the past two decades in epizootics of finfish and amphibians in several countries. The family *Iridoviridae* is known to comprise of five genera; two of which are responsible for the infection of invertebrates (*Iridovirus* and *Chloriridovirus*), while the other three are known to infect only ectothermic vertebrates (*Lymphocystivirus*, *Megalocytivirus*, and *Ranavirus*) (Jancovich et al., 2015a). Lymphocystiviruses and megalocytiviruses have been observed to be responsible for the infection of only fish, while ranaviruses infect fish, amphibians, as well as reptiles (Chinchar et al., 2011; Lesbarrères et al., 2012; Gray and Chinchar, 2015). Singapore grouper iridovirus (SGIV) and grouper iridovirus (GIV) are genetically related ranaviruses, that have been known to have negative impacts on grouper mariculture in Asia since the 1990s (Chua et al., 1994; Murali et al. 2002; Qin et al., 2003).

Iridoviral infections involving multiple systems have been reported in a range of freshwater food fish species

(Whittington et al., 1994; McGrogan et al., 1998; He et al., 2000), tropical freshwater ornamental fish (Armstrong and Ferguson, 1989; Anderson et al., 1993; Rodgers et al., 1997) and marine food fish species (Inouye et al., 1992; Bloch and Larsen, 1993; Chou et al., 1998; Matsuoka et al., 1996; Nakajima et al., 1998; Jung and Oh, 2000). In the past, several researchers identified distinct peculiar histopathological lesions associated with localized or systemic iridoviral infections of fish (Langdon and Humphrey, 1987; Langdon et al., 1986; Hetrick and Hedrick, 1993; Plumb et al., 1996).

There are more than 2000 fish farmers engaged in the culture of marine finfish (groupers) in Malaysia. Groupers are popular aquaculture fish species in Malaysia and account for more than 16 and 30 % by weight and value of total marine fish, respectively. Furthermore, nearly 15 % of the fish seeds are procured from the wild waters, government or private hatcheries. The remaining 85 % are sourced mainly from Taiwan and Thailand (Subramaniam, 1999). Grouper attains high market demand both in local and international markets and constitute the top menus for restaurants, hotels and resorts, especially during festive seasons. However, the supply of groupers from aquaculture is often limited due to diseases, which occur throughout the production cycle (Muroga, 2001; Hyatt and Whittington, 2002; Bondad-Reantaso et al., 2005; Harikrisnan et al., 2010). Recently, a disease outbreak occurred in grouper aquaculture in Sabah, Malaysia that resulted in heavy mortalities over a short period of time. The fish exhibited dark skin coloration and abnormal swimming behavior, suffered from skin lesions, hemorrhages and fin erosion. Based on the clinical signs in affected fish, the outbreak might be due to Iridoviridae; a viral disease that has been widely reported to cause high mortality among groupers (Chia et al., 2004; Eaton et al., 2007; Chinchar et al., 2008), and a range of other freshwater and marine fish species (Fauquet et al., 2005; Eaton et al., 2008; Murwantoko and Pratiwe, 2009). However, the presence of Iridoviridae in fish is difficult to detect because it can persist for a very long time in host cells without manifesting any detectable effects. As such, the host can become the asymptomatic carrier of the virus (Jeong et al., 2006). Hence, the ability to detect the carrier fish could help prevent future disease outbreak from occurring in aquaculture.

The aim of this research was to identify and define the pathology observed in PCR positive systemic iridoviral infections in naturally infected groupers using light microscopic examination of hematoxylin and eosin stained tissue sections.

Materials and methods

Collection of samples

The study was based on 150 grouper fish suspected with Iridoviral infection from cultured farms distributed throughout Peninsular Malaysia. The fish were brought to the Aquatic Animal Health Unit, Universiti Putra Malaysia, between 2012 and 2014. The cases were selected on the basis of histopathological evidence of systemic iridoviral infection. The size of the fish ranged between 6 and 10 cm by 20–30 cm. Moribund fish were transported to the laboratory on ice for initial diagnostic tests. Grouper species were selected because they have been reported to be susceptible to systemic iridoviral infection. The fish were sacrificed by pithing and visceral organs (kidney, spleen and liver) were removed and preserved in 10 % buffered formalin for histological studies.

Sample preparation for histopathology evaluation

The tissues of PCR positive groupers (liver, spleen, kidney, gill, heart, eye, and skin with ulcers) were fixed in 10 % buffered formalin solution for no less than 24 h. Tissue blocks sized 5 mm^3 were cut from fixed samples, processed, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E). The prepared slides were observed under light microscopy for the presence of lesions at various magnifications, and photomicrographs were taken using a camera (Leica DM LB2).

DNA extraction, PCR and gel electrophoresis

DNA was extracted from 30 to 50 mg of fish spleen using Universal All TM Genomic DNA Extraction Mini Kit for animal tissue (Yeastern Biotech Co, Taiwan). The tissue was cut and then placed in a 1.5-L micro-centrifuge tube and homogenized with a micro-pestle and DNA extraction was carried out as stated in the manufacturer's protocol (http://www.yeastern.com/updfile/ProductsFile/UniversAll%20English%20+%20Chinese.pdf). The purified DNA solution was stored at −20 °C until used.

Grouper iridovirus (GIV) was detected in fish DNA samples using a two-step commercial kit IQ2000 Iridovirus (Detection kit, Farming IntelliGene Tech. Corp, Taipei, Taiwan). Protocol for the polymerase chain reaction (PCR) was carried out according to the manufacturer's instructions. The protocol involves two primer sets for a nested PCR amplification; the first reaction mixture contained 7.5 μL of PCR Premix and 0.5 μL of IQzyme DNA Polymerase, to which was added 2 μL of DNA template and 5 μL of 6× loading dye. This reaction mixture was then used in the subsequent direct PCR for iridovirus, and nested PCR was carried out in 14 μL of reaction mixture containing 1 μL IQzyme DNA Polymerase to obtain a 25 μL final reaction volume. Amplification was carried out in the Eppendorf Mastercycler®pro (Vapo.Protect, USA) with 15 cycles comprising of an initial denaturation at 94 C for 2 min, followed by 15 cycles of amplification (94 °C for 20 s, 62 °C for 20 s, and 72 °C for 30 s) and extension for 30 s at 72 °C. The second PCR profile was performed at 94 °C for 20 s; 62 °C for 20 s; 72 °C for 30 s, with 30 repeat cycles

and extension at 72 °C for 30 s followed by a final extension for 5 min at 72 °C. PCR products were run on 2 % Agarose gel electrophoresis and stained with RedSafe™ Nucleic Acid Staining Solution (20,000×). Electrophoresis was carried out at 100 V for 60 min in 1× TBE buffer (Tris-borate). The positive bands were detected under UV illumination and photographed by an Alpha imager machine.

Results

Clinical signs

Here the clinical signs reported were abnormal swimming with spasm, decreased feeding, lethargy and darkening of the tail and fins with focal distension of the skin and pale gills, necrosis of fins, sloughing of epidermis, dermal ulceration, enlarged spleen, followed by red boils on the skin and red spots on the skin and head. Moribund fish with rapid opercula movements float in the water surface before eventually sinking to the bottom and dying.

Detection of grouper iridovirus (GIV) by PCR

From the 150 fish samples collected, 27 fish samples were identified to be positive for GIV. The results showed a single DNA band (between 226 and 450 bp), which was comparable with the positive control for iridoviral disease (Fig. 1).

Gross and histopathology lesion evaluation in infected groupers

Grossly, the groupers had ulceration on the operculum and close to the caudal fin with hemorrhages on the margin of the caudal fin. In some fish, there was necrosis of the caudal fin, sloughing of the epidermis, dermal ulceration and popeyes (Fig. 2a, b).

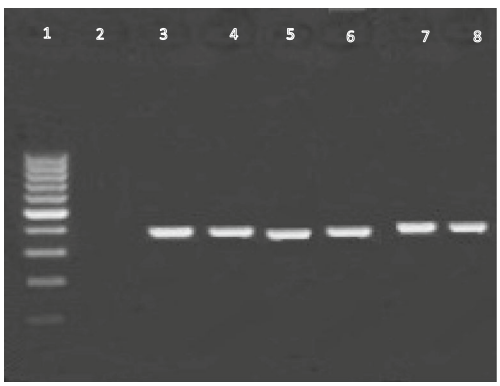

Fig. 1 Detection of GIV by PCR using grouper species of susceptible viral disease tissue DNA as template. *1* = Lader: 100 bp DNA ladder (Fermentas, Lithuania), *2* = −ve: negative control, *3* = +ve: positive control, *4–8* = samples

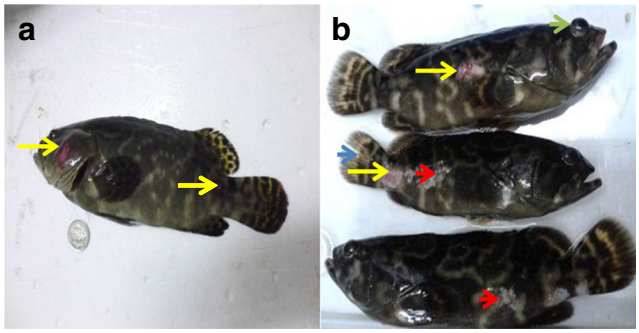

Fig. 2 Gross photograph of infected grouper fish showing **a** an ulceration on the operculum and close to the caudal fin (*yellow arrows*), **b** necrosis of the caudal fin (*blue arrow*), sloughing of the epidermis (*red arrow*), ulceration (*yellow arrow*), and popeyes (*green arrow*)

Histopathological changes associated with iridoviral infection were often detected in the liver, spleen, kidney, heart, and gills. The tissues examined showed various degrees of degeneration and necrosis. The epidermis, dermis, stomach and intestine were all affected by GIV infection. Infected fish showed evidence of vascular necrosis, hepatic edema, and hyperplasia of branchial epithelial cells with a fusion of secondary lamellae. Hemorrhage was found in the epidermis and dermis of most diseased fish, this suggests that those that survive the acute infection may develop an ulcerative syndrome, which becomes a source of infection to other fish.

In this study, we observed the most significant change in all affected fish to include the presence of basophilic, hypertrophied cells, frequently in significant numbers in several organs, with inclusion body and hyaline capsules being most dominant. Viral inclusion bodies were detected in the cytoplasm of endothelial cells, epithelial cells and leukocytes. Hypertrophied cells, with a pale foamy or intensely basophilic granular appearance, have been often visible, especially in the liver, splenic parenchyma and capsule, and in the renal glomerulus and interstitium. These cells were also seen in the pancreatic interstitium, and in the connective tissues of the body. They were usually detected in smaller numbers in the liver, and gills. Dark staining crescent-shaped cells were often visible around the hypertrophied cells. In addition, a thick hyaline capsule surrounding the hypertrophied fibroblast was observed in the cytoplasm (Fig. 3a).

In the liver, changes observed early in the course of the infection include sinusoidal necrosis, hepatocyte swelling, focal hepatocellular necrosis characterized by nuclear pyknosis. Multifocal areas of liquefactive necrosis were also seen. Inflammatory infiltration was occasionally seen around blood vessels. Lesions observed ranged from very small to extensive areas of cellular destruction. In advanced stages, there was evidence of progressive vacuolar degeneration, cloudy swelling, hypertrophy, apoptosis, and hyaline degeneration (Fig. 3a). In the gill, there was evidence of vacuolar degeneration, thickening of the basal membrane of the secondary lamellae or gill synechiae, separation of the secondary lamellae epithelium from pillar cells, hyperplasia, mononuclear infiltration, and clubbing (Fig. 3b).

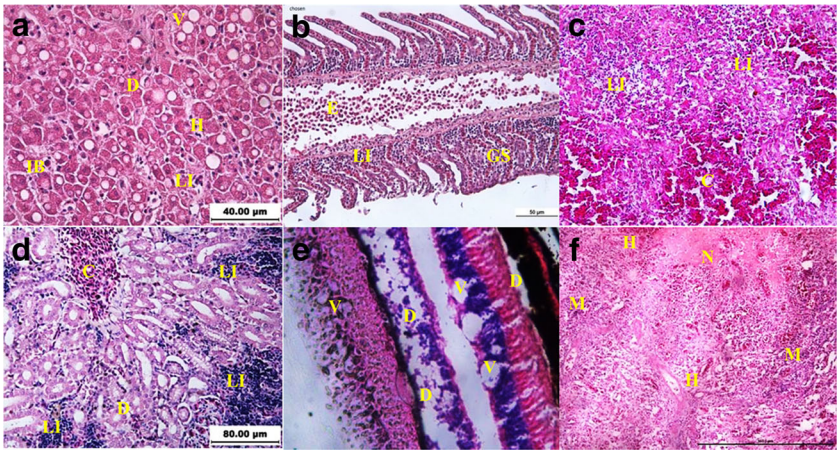

Fig. 3 Photomicrograph of tissue sections of grouper infected with GIV. **a** Liver showing vacuolar degeneration (*V*) of hepatocytes and cloudy swelling (*D*), hypertrophy of hepatocytes (*H*) with presence of inclusion bodies (*IB*) can also be seen. Note mild mononuclear leucocytic infiltration (*LI*) in the sinusoid cavity, H&E × 400; **b** gills showing matting of gill filaments (gill synechiae), mononuclear leucocytic infiltration (*LI*) and edema (*E*), H&E × 200; **c** spleen showing congestion of the red pulp (*C*) and leucocytic depopulation in the white pulp (*LI*), H&E × 200; **d** kidney showing congestion (*C*), leucocytic infiltration in the interstitium (*LI*), and degeneration of renal tubules (*D*), H&E × 400; **e** eyes showing vacuolation in the outer nuclear layer and ganglion cells (*V*), with detachment of the inner nuclear and epithelial layers (*D*), H&E × 1000; and **f** skin showing a wide area of necrosis (*N*), areas of hemorrhage (*H*) and mononuclear cellular infiltration (*M*), H&E × 100

In the spleen, early changes include multiple tiny foci of necrosis and focal cellular necrosis of the splenic white pulp. Diffused viral inclusion bodies were rarely seen in the spleen (Fig. 3c). A section from a grouper kidney was observed to have proximal tubular degeneration, mononuclear infiltration, congestion, increased bowman's capsular space. In acute infection, observations revealed focal necrosis, margination of nuclear chromatin, and pyknosis in tubular cells. Glomeruli were sometimes shrunken, and necrotic (Fig. 3d).

Histopathological characteristics of a grouper eye showed vacuolar degeneration in the outer nuclear layer, separation of the inner nuclear layer, vacuolar degeneration of the ganglion cells, and separation of pigment epithelium layer from the layer of rods and cones (Fig. 3e). A section of ulcerated grouper skin (Epidermis), showed mononuclear infiltration, a necrotic area, and a hemorrhagic area (Fig. 3f). The heart tissue showed muscle fibers necrosis and fibrosis with the presence of hemocytes. Occasionally, very small focal areas of necrosis with associated melanosis were detected in the heart. Other lesions observed include necrosis of the endothelium, pancreatic cells, stomach, and intestine; hemorrhages in the dermis, muscles, eyes; and apoptosis in some organs such as the liver and skin.

Discussion

This is the first histopathological study of grouper iridovirus infection in *Epinephelus* species in Malaysia. The pathology of GIV is similar to *Ranavirus*-induced pathology seen in other fish (Moody and Owens 1994), amphibians (Cullen et al., 1995), and reptiles (Ariel and Owens, 1997). Lesions are variable but internal organ necrosis is common. The existence of inclusion bodies (IBCs) and enlarged cells during routine histopathological evaluations is an important diagnostic indication of systemic iridoviral infections.

In this study, multifocal, random cutaneous hemorrhages, petechiation (pinpoint hemorrhages) or ecchymosis, swellings and ulcerations are the typical gross lesions found in the skin and fins, petechiation (pinpoint hemorrhages) or ecchymosis of the internal organs, particularly the mesonephros—the kidneys and liver–consists of masses of individual nodules (Langdon and Humphrey 1987; Langdon et al. 1988, Waltzek et al., 2014). Red swollen gills observed here were similar to the findings of Mao et al. (1999). Other lesions such as hemorrhages observed in most organs, fat bodies and swim bladder, and friable consistency of organs have been previously described by Zilberg et al. (2000) and Waltzek et al. (2014). Reddacliff and Whittington (1996) have given detailed descriptions of lesions due to epizootic hematopoietic necrosis virus (EHNV) *Ranavirus* in redfin perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*). The findings of this study showed that sick fish had a dark color, ceased to eat and were sometimes ataxic. Gross lesions such as an enlarged spleen, kidneys and abdomen, with hepatic foci were also observed. In a similar study, Zilberg et al. (2000) reported the presence of necrosis in the gastrointestinal (GI) mucosal epithelium, heart and gills of *Micropterus salmoides* experimentally infected with *Santee-Cooper Ranavirus*.

In the present study, the occurrence of hematopoietic tissue necrosis and those of the vascular endothelium, epithelial cells with hemorrhage and intracytoplasmic basophilic inclusion bodies were considered microscopic lesions in groupers as was previously reported (Reddacliff and Whittington, 1996; Cunningham et al., 2007; Allender et al., 2013b; Bayley et al.,

2013; Cheng et al., 2014; Waltzek et al., 2014). Necrotizing syndromes with similar clinical appearance have been reported in cultured catfish (*Ictalurus melas*) in France (Pozet et al., 1992), sheatfish (*Silurus glanis*) in Germany (Ahne et al., 1989), turbot (*Scophthalmus maximus*) in Denmark (Bloch and Larsen, 1993), and other fish species in Europe (Ariel et al., 1999; Tapiovaara et al., 1998).

The findings of this study showed that, the liver, spleen, and kidney (including pronephros and mesonephros) are usually the three organs affected in fatal cases. These tissues showed various degrees of inflammation and necrosis. Systemic necrotizing syndromes mainly included serious necrosis of the renal tubules and hematopoietic tissues, splenic and hepatic tissues. Previous related studies have reported similar pathologies in other fishes and amphibians infected with closely related ranaviruses, such as EHNV (Langdon and Humphrey, 1987; Langdon, 1989); Frog virus 3 (FV3) (Wolf et al., 1968; Essanl and Granoff, 1989); sheatfish iridovirus (Ogawa et al., 1990); catfish iridovirus (Pozet et al., 1992), and *Rana temporaria* iridovirus (Cunningham et al., 1996). These may be some significant pathological signs found in grouper species, which develop into a more chronic type of grouper iridovirus disease.

In this study, acute hematopoietic necroses were detected in the kidneys, which varied from cells that were individually infected. Infected cells showed characteristic basophilic intracytoplasmic inclusions identified as virus assembly sites. Another consistent and conspicuous change in infected grouper is the same as in rainbow trout and redfin perch, which is the existence of degenerated hematopoietic cells, basophilic debris, and fibrous material in the blood vessels that also Reddacliff and Whittington (1996) reported. The marked cellular inflammatory responses seen in the liver, kidney, spleen, gill, and heart could be either due to vascular endothelial damage from neighboring infected cells, a reaction to severe viremia, or cells containing centrarchid-type inclusion bodies as observed in iridoviral disease (Wolf, 1988a). When existing in melanomacrophages, these cells were fragmented, and it was possible that they were a factor in the spread of the virus within the host. The presence of these cells in different fish species with iridoviral infection suggests that they are either transitional stages in the virus assembly or represent activation of secondary hematopoietic centers. In addition, degenerative changes in the vascular endothelium as observed in the liver¸ spleen, kidney, gill, and heart, may be an indication that the virus is endotheliotropic. These findings are similar those observed following infection of fish with other ranaviruses (Cunningham et al., 1996; Jancovich et al., 1997; Bollinger et al., 1999, Gibson-Kueh et al., 2003).

In this research, basophilic and eosinophilic enlarged cells were seen in kidney, liver, and gills and it was one of the distinctive features of GIV infection. Enlarged cells have been reported in many confirmed iridovirus infections of fishes. Similar findings have been described for a number of fish iridovirus including African lampeye iridovirus (ALIV) from African lampeye, GIV from grouper (*Epinephelus malabaricus*), sea bass iridovirus (SBIV) from sea bass (Miyata et al., 1997), Red Sea bream iridovirus (RSIV)-like iridovirus from striped beakperch (*Oplegnathus fasciatus*) (Jung and Oh, 2000, Kim et al., 2002), and infectious spleen and kidney necrosis virus (ISKNV) from the mandarinfish (*Siniperca chuatsi*) (He et al., 2001). The presence of large cells has been reported in other unidentified fish iridoviruses or iridovirus-like infections. Previously, Bloch and Larsen (1993) reported a systemic infection in cultured turbot where the secondary lamella capillary was occluded by the virus infected cells.

Conclusion

Based on the histo-cytopathologic observations of tissue sections under light microscopy, the virus detected in this study exhibited characteristic features of grouper iridovirus GIV. Histopathological study of grouper iridovirus GIV infections is one of the methods to confirm diagnosis of the disease in fish. Interestingly, PCR detection of the viral NP confirmed the presence of the virus in infected fish that showed gross and histopathological lesions of GIV. This study has thus established the presence of GIV in Malaysian culture fish farms; however, more studies need to be conducted to know the impact of the disease in other associated fish species apart from groupers.

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Author contributions All authors contributed equally to this work and are responsible for the data presented herein.

Ethical considerations Grouper fish used for this study is not an endangered or extinct species, thus there was no provision for special approval. However, agreed consent was given by the concerned fish farmers to conduct the research using moribund fish from their farms.

Conflict of interest The authors declare that they have no conflict of interest.

References

Ahne W, Schlotfeldt HJ, Thomsen I (1989) Fish viruses isolation of an icosahedral cytoplasmic deoxyribovirus from sheatfish (*Silurus glanis*). J Vet Med 36:333–336

Allender MC, Mitchell MA, Torres T, Sekowska J, Riskell EA (2013b) Pathogenicity of frog virus 3-like virus in red-eared slider turtles at two environmental temperatures. J Comp Pathol 149:356–367

Anderson IG, Prior HC, Rodwell BJ, Harris GO (1993) Iridovirus-like virions in imported dwarf gourami (*Colisa lalia*) with systemic amoebiasis. Aust Vet J 70:66–67

Ariel E, Owens L (1997) Epizootic mortalities in tilapia *Oreochromis mossambicus*. Dis Aquat Org 29:1–6

Ariel E, Tapiovaara H, Olesen NJ (1999) Comparison of pike-perch (*Stizostedion lucioperca*), cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*) iridovirus isolates with reference to other piscine and amphibian iridovirus isolates. European Association of Fish Pathologists, VIII. International Conference on Diseases of Fish and Shellfish, Rhodes, Greece, 20–24 September

Armstrong RD, Ferguson HW (1989) Systemic viral disease of the chromide cichlid *Etroplus maculatus*. Dis Aquat Org 7:155–157

Bayley AE, Hill BJ, Feist SW (2013) Susceptibility of the European common frog *Rana temporaria* to a panel of ranavirus isolates from fish and amphibian hosts. Dis Aquat Org 103:171–183

Bloch B, Larsen JL (1993) An iridovirus-like agent associated with systemic infection in cultured turbot *Scophthalmus maximus* fry in Denmark. Dis Aquat Org 15:235–240

Bollinger TK, Mao J, Schock D, Brigham RM, Chinchar VG (1999) Pathology, isolation, and preliminary molecular characterization of a novel iridoviurs from tiger salamanders in Saskatchewan. J Wildl Dis 35:413–429

Bondad-Reantaso MG, Subasinghe RP, Arthur JR, Ogawa K, Chinabut S, Adlard R, Shariff M (2005) Disease and health management in Asian aquaculture. Vet Parasitol 132(3):249–272

Cheng K, Jones MEB, Jancovich JK, Burchell J, Schrenzel MD, Reavill DR, Imai DI, Urban A, Kirkendall M, Woods LW, Chinchar VG, Pessier AP (2014) Isolation of a Bohle-like iridovirus from boreal toads housed within a cosmopolitan aquarium collection. Dis Aquat Org 111(2):139–152

Chia CB, Chun YC, Yueh YL, Chan SL, Hung TH (2004) Histological, ultrastructural, and in situ hybridization study on enlarged cells in grouper Epinephelus hybrids infected by grouper iridovirus in Taiwan (TGIV). Dis Aquat Org 58:127–142

Chinchar VG, Hyatt A, Miyazaki T, Williams T (2008) Family Iridoviridae: poor viral relations no longer. Microbiol Immunol 328:123–170

Chinchar VG, Yu KH, Jancovich JK (2011) The molecular biology of frog virus 3 and other iridoviruses infecting cold-blooded vertebrates. Viruses 3(10):1959–1985

Chou HY, Hsu CC, Peng TY (1998) Isolation and characterization of a pathogenic iridovirus from cultured grouper (Epinephelus sp.) in Taiwan. 病研究 33(4):201–206

Chua FHC, Ng ML, Ng KL, Loo JJ, Wee JY (1994) Investigation of outbreaks of a novel disease, "sleepy grouper disease", affecting the brown-spotted grouper (*Epinephelus tauvina* Forskal). J Fish Dis 17:417–427

Cullen BR, Owens L, Whittington RJ (1995) Experimental infection of Australian anurans (Limnodynastes terraginae and Litona latopalmata) with Bohle iridovirus. Dis Aquat Org 23:83

Cunningham AA, Hyatt AD, Russell P, Bennett PM (2007) Emerging epidemic diseases of frogs in Britain are dependent on the source of ranavirus agent and the route of exposure. Epidemiol Infect 135: 1200–1212

Cunningham AA, Langton TES, Bennett PM, Lewin JF, Drury SEN, Gough RE, Macgregor SK (1996) Pathological and microbiological findings from incidents of unusual mortality of the common frog (*Rana temporaria*). Philos Trans R Soc Lond Ser B Biol Sci 351: 1539–1557

Eaton HE, Metcalf J, Brunetti CR (2008) Expression of frog virus 3 genes is impaired in mammalian cell lines. Virol J 5:83

Eaton HE, Metcalf J, Penny E, Tcherepanov V, Upton C, Brunetti CR (2007) Comparative genomic analysis of the family Iridoviridae: re-annotating and defining the core set of iridovirus genes. Virol J 4:11

Essanl K, Granoff A (1989) Amphibian and piscine iridovirus proposal for nomenclature and taxonomy based on molecular and biological properties. Intervirology 30:23–35

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (2005) Virus taxonomy. Eighth report of the international committee on taxonomy of viruses. Elsevier Academic Presss, USA, pp. 145–161

Gibson-Kueh S, Netto P, Ngoh-Lim GH, Chang SF, Ho LL, Qin QW, Ferguson HW (2003) The pathology of systemic iridoviral disease in fish. J Comp Pathol 129(2):111–119

Gray MJ, Chinchar VG (2015) Introduction: history and future of ranaviruses. In: *Ranaviruses*. Springer International Publishing, p 1–7

Harikrisnan R, Chellam B, Moon SH (2010) Molecular studies, disease status and prophylactic measures in grouper aquaculture: economic importance, diseases and immunology. Aquaculture 10:1–14

He JG, Deng M, Weng SP, Li Z, et al. (2001) Complete genome analysis of the mandarinfish infectious spleen and kidney necrosis iridovirus. Virology 291:126–139

He JG, Wang SP, Zeng K, Huang ZJ, Chan SM (2000) Systemic disease caused by an iridoviruslike agent in cultured mandarinfish (*Siniperca chuatsi* Bailewsky) in China. J Fish Dis 23:219–222

Hetrick FM, Hedrick RP (1993) New viruses described in finfish from 1988 to 1992. In: Faisal M, Hetrick FM (eds) Annual review of fish diseases, vol 3. Pergamon Press, London, pp. 187–207

Hyatt AD, Whittington RJ (2002) Ranaviruses of fish, amphibians and reptiles: diversity and the requirement for revised taxonomy. Dis Asian Aquat V:155–170

Inouye K, Yamano K, Maeno Y, Nakajima K, Matsuoka M, Wada Y, Sorimachi M (1992) Iridovirus infection of cultured red sea bream, *Pagrus major*. Fish Pathol 27:19–27

Jancovich JK, Davidson EW, Morado JF, Jacobs BL, Collins JB (1997) Isolation of a lethal virus from the endangered tiger salamander *Ambystoma tigrinum stebbinsi*. Dis Aquat Org 31:161–167

Jancovich JK, Steckler N, Waltzek TB (2015a) Ranavirus taxonomy and phylogeny. In: Gray MJ, Chinchar VG (eds) Ranaviruses: lethal pathogens of ectothermic vertebrates. Springer, New York

Jeong JB, Jun LY, Park KH, Kim KH, Chung JK, Komisar JL, Jeong HD (2006) Asymptomatic iridovirus infection in various marine fishes detected by a 2-step PCR method. Aquaculture 255:30–38

Jung SJ, Oh MJ (2000) Iridovirus-like infection associated with high mortalities of striped beakperch, *Oplegnathus fasciatus* (Temminck et Schlegel) in southern coastal areas of the Korean peninsula. J Fish Dis 23:223–226

Kim YJ, Jung SJ, Choi TJ, Kim HR, Rajendran KV, Oh MJ (2002) PCR amplification and sequence analysis of irido-like virus infecting fish in Korea. J Fish Dis 25(2):121–124

Langdon JS (1989) Experimental transmission and pathogenicity of epizootic haematopoietic necrosis virus (EHNV) in redfin perch, *Perca fluviatilis* L., and 11 other teleosts. J Fish Dis 12(4):295–310

Langdon JS, Humphrey JD, Williams LM (1988) Editorial responsibility: managing editor outbreaks of EHNV-11ke lndovlrus in cultured rainbow trout, Salmo Gairdneri Richardson in Australia. J Fish Dis 11:93–96

Langdon JS, Humphrey JD (1987) Epizootic haematopoietic necrosis, a new viral disease in redfin perch, *Perca fluviatilis* L, in Australia. J Fish Dis 10:289–297

Langdon JS, Humphrey JD, Williams LM, Hyatt AD, Westbury HA (1986) First virus isolation from Australian fish: an iridovirus-like pathogen from redfin perch, *Perca fluviatilis* L. J Fish Dis 9:263–268

Lesbarrères D, Balseiro A, Brunner J, Chinchar VG, Duffus A, Kerby J, Gray MJ (2012) Ranavirus: past, present and future. Biol Lett 8(4): 481–483

Mao JD, Green E, Fellers G, Chinchar VG (1999) Molecular characterization of iridoviruses isolated from sympatric amphibians and fish. Virus Res 63:45–52

Matsuoka S, Inouye K, Nakajima K (1996) Cultured fish species affected by red sea bream 118 S. Gibson-Kueh et al. iridoviral disease from 1991 to 1995. Fish Pathol 31:233–234

McGrogan DG, Ostland VE, Byrne PJ, Ferguson HW (1998) Systemic disease involving an iridoviruslike agent in cultured tilapia, *Oreochromis niloticus* L.—a case report. J Fish Dis 21:149–152

Miyata M, Matsuno K, Jung SJ, Danayadol Y, Miyazaki T (1997) Genetic similarity of iridoviruses from Japan and Thailand. J Fish Dis 20: 127–134

Moody NJG, Owens L (1994) Experimental demonstration of the pathogenicity of a frog virus, Bohle iridovirus, for a fish species, barramundi *Lates calcarifer*. Dis Aquat Org 18:95–102

Murali S, Wu MF, Guo IC, Chen SC, Yang HW, Chang CY (2002) Molecular characterization and pathogenicity of a grouper iridovirus (GIV) isolated from yellow grouper, *Epinephelus awoara* (Temminck & Schlegel). J Fish Dis 25(2):91–100

Qin QW, Chang SF, Ngoh-Lim GH, Gibson-Kueh S, Shi C, Lam TJ (2003) Characterization of a novel ranavirus isolated from grouper *Epinephelus tauvina*. Dis Aquat Org 53(1):1–9

Muroga K (2001) Viral and bacterial diseases of marine fish and shellfish in Japanese hatcheries. Aquaculture 202(1):23–44

Murwantoko HCR, Pratiwe R (2009) Cloning and sequence analysis of capsid protein gene of iridovirus Indonesian isolates. Indian J Biotechnol 14:1117–1123

Nakajima K, Inouye K, Sorimachi M (1998) Viral diseases in cultured marine fish in Japan. Fish Pathol 33:181–188

Ogawa M, Ahne W, Fischer-Scherl T, Hoffmann RW, Schlotfeldt HJ (1990) Pathomorphological alterations in sheatfish fry *Silurus glanis* experimentally infected with an iridovirus-like agent. Dis Aquat Org 9:187–191

Plumb JA, Grizzle JM, Young HE, Noyes AD (1996) An iridovirus isolated from wild largemouth bass. J Aquat Anim Health 8:265–270

Pozet F, Morand M, Moussa A, Torhy C, De Kinkelin P (1992) Isolation and preliminary characterization of a pathogenic icosahedral deoxyribovirus from the catfish, *Ictalurus melas*. Dis Aquat Org 14:35–42

Reddacliff LA, Whittington RJ (1996) Pathology of epizootic haematopoietic necrosis virus (EHNV) infection in rainbow trout (*Oncorhynchus mykiss* Walbaum) and redfin perch (Perca fluviatilis L.). J Comp Pathol 115:103–115

Rodgers HD, Kobs M, Macartney A, Frerichs GN (1997) Systemic iridovirus infection in freshwater angelfish, *Pterophyllum scalare* Lichtenstein. J Fish Dis 20:69–72

Subramaniam K (1999) Grouper aquaculture development in Malaysia

Tapiovaara H, Olesen NJ, Linden J, Rimaila-Parnanen E, Von Bonsdorff C-H (1998) Isolation of an iridovirus from pikeperch *Stizostedion lucioperca*. Dis Aquat Org 32:185–193

Waltzek TB, Miller DL, Gray MJ, Drecktrah B, Briggler JT, MacConnell B, Hudson C, Hopper L, Friary J, Yun SC, Malm KV, Weber ES, Hedrick RP (2014) Expansion of the host range of frog virus 3 into hatchery-reared pallid sturgeon *Scaphirhynchus albus*. Dis Aquat Org 111:219–227

Whittington RJ, Philbey A, Reddacliff GL, MacGown AR (1994) Epidemiology of epizootic haematopoietic necrosis virus (EHNV) infection in farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum): findings based on virus isolation, antigen capture ELISA and serology. J Fish Dis 17:205–218

Wolf K (1988a) Fish viruses and viral diseases. Cornell University Press, Ithaca

Wolf K, Bullock GL, Dunbar CE, Quimby MC (1968) Tadpole edema virus: a viscerotropic pathogen for anuran amphibians. J Infect Dis 118:253–262

Zilberg D, Grizzle JM, Plumb JA (2000) Preliminary description of lesions in juvenile largemouth bass injected with largemouth bass virus. Dis Aquat Org 39:143–146