UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF GROUPER IRIDOVIRUS ISOLATED FROM GROUPER (Epinephelus spp.) IN PENINSULAR MALAYSIA

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FPV 2016 34

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By

MARYAM HAZERI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

October 2016

DEDICATION

Dedicated to my late father, ABBAS (Peace be upon him)

To my dearest and lovely mother, Mahijan

And my sister, Mojgan

who always give me their unlimited support, love, patience and understanding.

I am truly thankful for having you both in my life and in return would like to record my deepest expression of love to you.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

CHARACTERIZATION OF GROUPER IRIDOVIRUS ISOLATED FROM GROUPER (*Epinephelus* spp.) IN PENINSULAR MALAYSIA

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Chairman : Associate Professor Hassan Hj Mohd Daud, PhD
Faculty : Veterinary Medicine

Iridovirus infection in marine cultured fishes had caused serious mortalities in Southeast Asian countries especially in marine fishes farmed in Taiwan, Singapore, and Thailand due to grouper iridovirus (GIV) and the impact and extent of this disease is unknown hitherto in Malaysia. This is due to lack of knowledge on the host range, geographical distribution and the differences between strains if any. Hence to elucidate this gap, OIE reference polymerase chain reaction assay was utilized to detect the presence of grouper iridovirus in farmed grouper from Peninsular Malaysia. This current study aimed to examine the histopathological changes, using in-house design primers, sequence analysis of major capsid protein (MCP) gene, isolation of iridovirus in BF-2 and SSN-1 cell lines, biophysical and biochemical characterization and SDS-PAGE for characterization and comparing with other reference nucleotide sequences acquired from GenBank, and experimental infectivity study in red hybrid tilapia. A total of 150 hybrid grouper fish and coral trout samples were collected. Of these, GIV was detected in 27 hybrid grouper fish and coral trout samples and they were asymptomatic and or with mild non-specific lesion. Sequence analysis of MCP gene showed that the strain detected in this study was closely related to the reference of GIV and Ranavirus in an emerging disease which has been causing mortalities in grouper culture farms in Peninsular Malaysia. In addition, phylogenetic analysis of MCP gene revealed that the reference GIV and Ranavirus which were obtained from GenBank and all other strains that were detected in this study were included in genotype 1.

Clinical samples were collected from diseased grouper juveniles suspected of Iridovirus infection from marine floating cage fish farms. Some of the fish showed darkening of the body, uncoordinated swimming and skin ulcers. Microscopic examinations of the infected tissues showed severe necrosis with large intracytoplasmic inclusions, vacuolated cytoplasm and degenerated nuclei with marginated chromatin in different tissues.

The grouper iridovirus (GIV) was isolated in two fish cell lines i.e. BF-2 and SSN-1. The results showed that BF-2 cell line was more susceptible than SSN-1 to GIV with typical cytopathic effect (CPE) manifesting mainly as cells rounding-up, severe vacuolation and, progressive plaques of rounded-up cells within 3-5 days and complete detachment within 7 dpi.

Biophysical and biochemical characterization of GIV isolate were determined by heat treatment, UV irradiation and the stability under effect of chemical disinfectants (ether, formalin, iodine) and pH. The GIV isolate showed susceptibility to heat treatment at 56℃, UV irradiation, different pH ranging between 2 to 11, treatment with 2% formalin and iodine.

Since all the infected fish appeared healthy, there was a concern over possible transmission of asymptomatic GIV infection in freshwater fish species. To clarify this, an *in vivo* infection was conducted to investigate the possible susceptibility of red hybrid tilapia using 0.1 ml ($10^{8.5}$ $TCID_{50}$/0.1 ml) inoculum originated from BF-2 cell cultures via intraperitoneal injection. The GIV was able to infect tilapia fish within first week of injection, but the infected fish were asymptomatic. In conclusion, GIV pose serious risk to the Malaysian aquaculture industry as this virus can spread without any sign of disease.

In summary, the extent of GIV infection in grouper fish farms, which includes information on pathogenicity and geographical distribution in Peninsular Malaysia have been elucidated in this study. This baseline information is essential to mitigate the spread of this disease. Present study also confirmed the infectivity of 0.1 ml ($10^{8.5}$ $TCID_{50}$/0.1 ml) of BF-2 cell filtrate to red hybrid tilapia by IP injection. Integration of histopathological results such as confirmatory PCR-assays, sequence analysis of MCP, phylogenetic analysis, isolation virus on BF-2 and SSN-1 cell lines, and SDS-page of virus protein are essential in the diagnosis of grouper Iridovirus infection.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENCIRIAN GROUPER IRIDOVIRUS (GIV) DIPENCILKAN DARIPADA KERAPU, (*Epinephelus* spp.) DI MALAYSIA

Oleh

MARYAM HAZERI

Oktober 2016

Pengerusi : Profesor Madya Hassan Hj Mohd Daud, PhD
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Jangkitan iridovirus pada ternakan ikan marin menyebabkan kematian yang serius di negara-negara Asia Tenggara terutamanya Taiwan, Singapore, dan Thailand disebabkan oleh iridovirus kerapu (GIV) dan kesan serta tahap penyakit tidak diketahui sehingga kini di Malaysia. Ini disebabkan kekurangan dari segi pengetahuan mengenai julat perumah. taburan geografi dan perbezaan antara strain. Oleh itu, untuk menunjukkan perbezaan ini, penjujukan rantaian polimerase rujukan OIE digunakan untuk mengesan kehadiran grouper iridovirus pada ternakan grouper dari Semenanjung Malaysia. Kajian ini memfokuskan untuk mengkaji perubahan histopatologi, menggunakan primer dalaman, analisis jujukan gen protein kapsid utama (MCP), pemencilan iridovirus dalam BF-2 dan SSN-1 kultur sel, pencirian biofizikal dan biokimia serta SDS-PAGE untuk pencirian dan pembezaan dengan jujukan nukleotida rujukan lain daripada GenBank, dan jangkitan secara eksperimen dalam tilapia hibrid. Sejumlah 150 sampel ikan kerapu hibrid dan kerapu bara diambil. GIV dikesan pada 27 sampel grouper hibrid dan kerapu bara dan ia adalah asimptomatik dan/ atau dengan lesi tidak spesifik yang lemah. Analisis jujukan genMCP menunjukkan strain yang dikesan dalam kajian ini sangat berkait rapat dengan GIV rujukan dan Ranavirus pada penyakit bangkit yang menyebabkan kematian di ladang ternakan grouper di Semenanjung Malaysia. Tambahan pula, analisis filogenetik gen MCP menunjukkan GIV rujukan dan Ranavirus yang diperolehi daripada GenBank dan semua strain lain daripada kajian ini termasuk di dalam genotip 1.

Sampel klinikal diperolehi daripada kerapu juvenil yang sakit disyaki disebabkan oleh jangkitan iridovirus daripada ladang sangkar ikan terapung. Sesetengah ikan menunjukkan badan yang gelap, berenang tidak betul dan ulser kulit. Ujian mikroskopik pada tisu yang dijangkiti menunjukkan nekrosis yang teruk dengan rangkuman intrasitoplasma besar, sitoplasma tervakuol dan kemerosotan nukleus dengan kromatin bermarginat dalam tisu yang berbeza.

Iridovirus kerapu (GIV) dipencilkan dalam dua kultur sel iaitu BF-2 dan SSN-1. Hasil menunjukkan sel BF-2 lebih rentan kepada GIV berbanding SSN-1 dengan kesan sitopatik tipikal (CPE) yang jelas seperti sel menjadi bulat, pemvakuolan yang teruk dan plak progresif sel yang membulat diantara 3-5 hari dan penanggalan yang lengkap diantara 7 hari selepas jangkitan.

Pencirian biofizikal dan biokimia isolat GIV ditentukan oleh perawatan haba, iridasi UV dan kestabilan bawah kesan disinfektan kimia (eter, formalin, iodin) serta pH. Isolat GIV menunjukkan kerentanan kepada perawatan pada56℃, iridasi UV, perbezaan pH di antara 2 hingga 11, perawatan dengan 2% formalin dan iodin.

Kerana semua ikan yang terjangkit adalah sihat, ini memberi perhatian keatas kemungkinan penyebaran jangkitan GIV secara asimptomatik dalam spesies ikan air tawar. Untuk membuktikannya, jangkitan secara *in vivo* dilakukan untuk menyiasat kemungkinan kerentanan tilapia merah hibrid menggunakan inokulum 0.1 ml ($10^{8.5}$ $TCID_{50}$/0.1 ml) daripada sel kultur BF-2 melalui suntikan intraperitoneum. GIV mampu menjangkiti ikan tilapia dalam masa seminggu pertama suntikan, tetapi ikan tersebut menunnjukkan tanda-tanda asimptomatik. Kesimpulannya, GIV memberi risiko yang serius kepada industry akuakultur Malaysia kerana virus ini boleh merebak tanpa sebarang tanda-tanda penyakit.

Secara keseluruhannya, had jangkitan GIV pada kerapu di ladang ternakan, termasuk maklumat kepatogenan dan taburan geografi di Semenanjung Malaysia telah dihuraikan pada kajian ini. Dasar maklumat ini adalah penting untuk mengurangkan penyebaran penyakit. Kajian ini juga mengesahkan kejangkitan 0.1 ml ($10^{8.5}$ $TCID_{50}$/0.1 ml) turasan sel BF-2 pada tilapia merah hibrid melalui suntikan IP. Integrasi hasil histopatologi seperti pengesahan asai PCR, analisis jujukan MCP, analisis filogenetik, pemencilan virus pada BF-2 dan SSN-1 sel kultur, dan SDS-PAGE protein virus adalah penting dalampendiagnosan jangkitan iridovirus kerapu.

ACKNOWLEDGEMENTS

First and foremost, I give my greatest thanks to God for giving me the wisdom, ability, health and endurance to complete this degree.

I would like to express the deepest appreciation to my supervisor **Assoc. Prof. Dr Hassan Hj Mohd Daud** for his encouragement, ever-lasting support, guidance and supervision of this thesis. You have been a tremendous mentor for me. Without his supervision and constant help this dissertation would not have been possible.

I would also like to appreciate my other committee members: **Profesor Dr Abdul Rahman Omar** and **Assoc. Prof. Dr Zeenathul Nazariah Binti Allaudin** for their encouragement, useful comments, and challenging questions. I would also like to express thanks to the technical staff of Faculty of Food Science and Technology in Food Biochemistry and Engineering Laboratories. A very special thank you goes to **Mr. Mohd Jamil Samad** and **Mr Saipuzaman Ali** for being so supportive and warm welcome.

The most importantly, I take pleasure in expressing my gratitude to my father, **Abbas** (Peace be upon him) and my mother **Mahijan**, who always encouraged me to obtain higher education. They bore me, raised me, supported me, taught me, and loved me. To them I dedicate this thesis. I'm so lucky to have them be my parents.

A special thanks to my caring, loving, and supportive sister **Mojgan**. Words cannot express how grateful I am to her for all of the sacrifices that you've made on my behalf. She has been my best friend and a great companion, loved, supported, encouraged, entertained, and helped me get through this agonizing period in the most positive way.

I want to thank my beloved sister, **Mahvash**, two of my brothers, **Mahmoud** and **Mohamnmad Reza**, and my cousine **Abdolhamid Chalabi** who have always stimulated me to stay open-minded and to keep on doing the best I could. I would also like to thank my other siblings for all emotional support. Thanks for being with me.

Lastly, my special thanks to my best friends **Simin, Elham, Mahdokht, Parastoo, Hanie, Mina Babrkhani,** and **Yusuf Abba** for helping me get through the difficult times, and for all the emotional support, camaraderie, entertainment, and caring they provided.

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

µL	Micro liter
µM	Micromolar
ATV	Ambystoma tigrinum virus
BF	Bluegill fry
Bp	Base pairs
CPE	Cytopathic effect
dH_2O	Distilled water
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
Dpi	Day post infection
EHNV	epizootic haematopoietic necrosis virus
EtBr	Ethidium bromide
FBS	Fetal bovine serum
FV3	Frog virus 3
GIV	grouper iridovirus
H	hour (s)
HBSS	Hanks' balanced salt solution
H&E	Haematoxylin and Eosin
IBC(s)	Inclusion body-bearing cell(s)
ICTV	International Committee on Taxonomy of Viruses
IP	Intraperitoneal
KDa	Kilo Dalton
MCP	Major capsid protein

Min	minute (s)
OIE	Office International des Epizooties
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
RBC	Red Blood Cells
RGV	Rana grylio virus
RSIV	Red sea bream iridovirus
SGIV	Singapore grouper iridovirus
sp.	species
SSN	striped snakehead
TCID	Tissue culture infective dose
TFV	tiger frog virus
TEM	Transmission electron microscope
TL	Total Lysis
µl	Micro liter
µm	Micro meter

CHAPTER 1

INTRODUCTION

The aquaculture industry is threatened by many infectious diseases, and viruses, bacteria, fungi, and metazoan parasites are the most frequent agents (Meyer, 1991; Whittington & Chong, 2007). Among these the most harmful are viruses of the *Iridoviridae* family, which have been found to be responsible for serious rates of mortality that have resulted in substantial economic losses to the aquaculture industry (Chao et al., 2002). Williams (1996) and Xeros (1954) have reported that Claude Rivers was the first to detect the iridovirus in March 1954 by isolating it from crane fly larvae (*Tipula paludosa*) that exhibited glowing patches that were blue in color. Since then there have been several investigations of the *Iridoviridae,* with the majority of them focusing on iridescent viruses (Xerox, 1954). In the case of amphibian iridovirus, initial research began when an isolate linked to with renal carcinoma in the leopard frog (*Rana pipiens*) was discovered by Granoff et al. (1966).

It has been found that for the past two decades, infectious iridoviruses have been a significant agent of viral diseases in fish (Williams et al., 2005), in European countries, the US and also in East and Southeast Asian countries including Hong Kong, Korea, Malaysia, Philippines, Singapore and Thailand (Mahardika et al., 2004; Do et al., 2005; Jeong et al., 2006a). According to the 9th Report of the International Committee on Taxonomy of Viruses (ICTV), the family *Iridoviridae* comprises five genera, *Iridovirus*, *Chloriridovirus*, *Ranavirus*, *Lymphocystisvirus* and *Megalocytivirus* (Jancovich et al., 2012), with *Megalocytivirus* receiving most attention from researchers because of its wide host range and the high mortality it causes in the aquaculture industry (Dong et al., 2011).

Viruses such as frog virus 3 (FV3; *Ranavirus*) (Tan et al., 2004), soft-shelled turtle iridovirus (STIV; *Ranavirus*) (Huang et al., 2009), tiger frog virus (TFV; *Ranavirus*) (He et al., 2002), epizootic haematopoietic necrosis virus (EHNV; *Ranavirus*) (Jancovich et al., 2010), Ambystoma tigrinum virus (ATV; *Ranavirus*) (Jancovich et al., 2003), grouper iridovirus (GIV; *Ranavirus*) (Tsai et al., 2005), Singapore grouper iridovirus (SGIV; *Ranavirus*) (Song et al., 2004), are included as belonging to this genus but have not received approval as a virus species. There is a likelihood that host range of Ranaviruses animals and their products feature significantly in these epizootics occurrences (Hedrick & McDowell, 1995; Plumb & Zilberg, 1999a; Grant et al., 2005; Schramm & Davis, 2006; Deng et al., 2011; George et al., 2014).

Ranaviruses have attracted the attention of many researchers because of the serious harm they can cause to farmed and wild fish as well as amphibians (Pozet et al., 1992; Cullen & Owens, 2002; Bigarre et al., 2008). It has been recorded that the ranavirus of Epizootic Haematopoietic Necrosis Virus (EHNV) from Australia was the first iridovirus responsible for epizootic mortality in finfish (Langdon et al., 1986b). On

the other hand, on many occasions, there have been isolations of FV3-like ranaviruses from fishes in captivity.

The incidence of infectious disease is a major problem in hatchery production and an economic threat to the rearing of this fish which is of significant economic importance as a cultured marine fish in many Asian countries (Chi, 1997; Lai et al., 2001a, b). The threat to this industry is due to the fact that Iridoviruses are among the most significant viral pathogens in grouper, especially at the fry and fingerling stages (Lai et al., 2000, 2003). Singapore grouper iridovirus (SGIV) and grouper iridovirus (GIV) are genetically different but related ranaviruses that have had a negative impact on grouper mariculture in Asia since last decade of the 20th century (Chua et al., 1994; Murali et al., 2002; Qin et al., 2003).

Diagnostic methods such as histology and transmission electron microscope (TEM) have limited specificity and unable to detect low numbers of the viruses. In addition, PCR and qPCR cannot be carried out in resource limited laboratories and field due to the requirement of sophisticated equipment. A highly specific, simple, and inexpensive diagnostic tool is required to detect the presence of GIV to monitor the virus and ensure a healthy development of the grouper fish-farm industry. Thus, this study was carried out with the following hypotheses and objectives which will be to establish a better understanding of GIV that infects farmed grouper fish and red hybrid tilapia fish farmed from Peninsular Malaysia.

1.1 Hypotheses of the study:

Hypothesis 1. There will be variation amongst the iridovirus isolates from Peninsular Malaysia and the different grouper iridovirus (GIV) strains infecting grouper (*Epinephelus* sp.).

Hypothesis 2. Asymptomatic GIV infection could be induced in Red hybrid tilapia (*Oreochromis* sp.) by intraperitoneal infection (IP).

1.2 Objectives of the study:

i. To detect and determine the infectivity of Grouper Iridovirus (GIV) from farmed grouper using commercial cell lines, BF-2 and SSN-1.
ii. To characterize the cell culture-adapted virus biophysically and biochemically.
iii. To determine the molecular characteristic of the cell culture-adapted virus and infer the phylogenetic relationship of the isolates based on conserved MCP gene.
iv. To determine the pathogenicity of GIV in experimentally infected non-marine fish following IP infection.

BIBLIOGRAPHY

Agius, C. (1980). Phylogenetic development of melano-macrophage centres in fish. *Journal of Zoology* 191: 11–31.

Agius, C. (1985). The melano-macrophage centres in fish: a review. In Fish Immunology, ed. M.J. Manning and M.F. Tatner, *Academic Press* pp. 85–105. London.

Agius, C. and Roberts, R.J.(2003). Melano-macrophage centres and their role in fish pathology. *Journal of Fish Diseases* 26: 499-509.

Agius, C., Mangunwiryo, H., Johnson, R.H.and Smail, D.A. (1982). A more sensitive technique for solating infectious pancreatic necrosis virus from asymptomatic carrier rainbow trout, Salmo gairdneri ichardson. *Journal of Fish Diseases*, 5, 285-292.

Ahne, W. (1994). Viral infections of aquatic animals with special reference to Asian aquaculture. *Annual review of fish diseases*, *4*, 375-388.

Ahne, W., Schlotfeldt, H. J., and Thomsen, I. (1989). Fish viruses: isolation of an icosahedral cytoplasmic deoxyribovirus from sheatfish (Silurus glanis). *Journal of Veterinary Medicine, Series B*, *36*(1-10), 333-336.

Ahne, W., Ogawa, M. and Schlotfeldt, H. J. (1990). Fish viruses: transmission and pathogenicity of an icosahedral cytoplasmic deoxyribovirus isolated from sheatfish (Silurus glanis). *Journal of Veterinary Medicine, Series B*, *37*(1-10), 187-190.

Anhe, W., Schlotfeldt, H. J. and Ogawa, M. (1991). Iridovirus infection of adult sheatfish (Silurus glanis). *Bulletin of the European Association of Fish Pathologists*, *11*(3), 97-98.

Ahne, W., Bremont, M., Hedrick, R. P., Hyatt, A. D., and Whittington, R. J.(1997). Iridoviruses associated with epizootic haematopoietic necrosis (EHN) in aquaculture. *World Journal of Microbiology and Biotechnology*, *13*(4), 367-373.

Allender, M.C. (2012). Characterizing the epidemiology of ranavirus in North American chelonians: diagnosis, surveillance, pathogenesis, and treatment. Ph.D. Thesis, Department of Veterinary Clinical Medicine, University of Illinois at Urbana-Champaign, Urbana, p 219.

Byrd, J. (2013). Prevalence, clinical signs, and natural history characteristics of frog virus 3-like infections in eastern box turtles (Terrapene carolina carolina). *Herpetological Conservation and Biology*, *8*(2), 308-320.

Anderson, I. G., Prior, H. C., Rodwell, B. J. and Harris, G. O. (1993). Iridovirus-like virions in imported dwarf gourami (*Colisa lalia*) with systemic amoebiasis. *Australian Veterinary Journal,* 70, 66–67.

Allender, M.C., Mitchell, M. A., Torres, T., Sekowska, J., and Driskell, E. A. (2013). Pathogenicity of frog virus 3-like virus in red-eared slider turtles (Trachemys scripta elegans) at two environmental temperatures. *Journal of comparative pathology*, *149*(2), 356-367.

Allender, M.C., Fry, M.M., Irizarry, A.R., Craig, L., Johnson, A.J., and Jones, M. (2006). Intracytoplasmic inclusions in circulating leukocytes from an eastern box turtle (Terrapene carolina carolina) with iridoviral infection. *Journal of Wildlife Diseases*, 42:677-684.

Anonymous, (2006). Council directive 2006/88/EC of 24 October 2006 on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals. *Official journal of the European Union* L 328, p 14-56.

Argot, J., and Malsberger, R. G. (1972). Intracellular replication of infectious pancreatic necrosis virus. *Canadian journal of microbiology*, 18(6), 865-867.

Ariel, E. (1997). Pathology and serological aspects of Bohle iridovirus infections in six selected water-associated reptiles in North Queensland. PhD thesis, James Cook University, Queensland

Ariel, E., and Bang J.B. (2009a). Challenge studies of European stocks of redfin perch, Perca fluviatilis L., and rainbow trout, Oncorhynchus mykiss (Walbaum), with epizootic haematopoietic necrosis virus. *Journal of Fish Diseases* 32:1017-1025.

Ariel, E., and Owens, L. (1997). Epizootic mortalities in tilapia *Oreochromis mossambicus* . *Diseases of Aquatic Organisms*, 29:1–6.

Ariel, E., Tapiovaara, H., and Olesen, N.J. (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.

Ariel, E., Holopainen, R., Olesen, N.J., and Tapiovaara, H. (2010). Comparative study of ranavirus isolates from cod (*Gadus morhua*) and turbot (*Psetta maxima*) with reference to other ranaviruses. *Archives of Virology* 155, 261–271.

Ariel, E., Kielgast, J., Svart, H.E., Larsen, K., Tapiovaara, H., Jensen, B.B., and Holopainen, R. (2009b). Ranavirus in wild edible frogs *Pelophylax kl. esculentus* in Denmark. *Diseases of Aquatic Organisms*, 85:7-14.

Armstrong, R.D., and Ferguson, H.W. (1989). Systemic viral disease of the chromide cichlid Etroplus maculatus. *Diseases of Aquatic Organisms*, 7:155–157.

Aubertin, A. M. (1991). Family Iridoviridae. In classifi cation and nomenclature of viruses (edited. by Francki, R. I. B., C. M. Fauquet, D. L. Knudson and F. Brown). Classification and nomenclature of viruses. *Springer, Wien* New York, pp. 132-136.

Aubertin, A.M., Hirth, C. and Travo, C. (1973). Preparation and properties of an inhibitory extract from frog virus 3 particles. *Journal of Virology* 11:694–701

Bailey, A. and Feist, S. (2011). Transmission of ranavirus in the common frog Rana temporaria by indirect cohabitation In abstracts of the 15th EAFP International Conference on Diaseases of Fish and Shellfish. 12-16th September 2011, Split, p 349.

Balseiro, A., Dalton, K..P., del Cerro, A., Marquez, I., Cunningham, A.A., Parra, F., Prieto, J.M. and Casais, R. (2009). Pathology, isolation and molecular characterisation of a ranavirus from the common midwife toad Alytes obstetricans on the Iberian Peninsula. *Diseases of Aquatic Organisms*, 84:95-104.

Balseiro, A., Dalton, K. P., Del Cerro, A., Márquez, I., Parra, F., Prieto, J. M., and Casais, R. (2010). Outbreak of common midwife toad virus in alpine newts (Mesotriton alpestris cyreni) and common midwife toads (Alytes obstetricans) in Northern Spain: A comparative pathological study of an emerging ranavirus. *The Veterinary Journal*, 186(2), 256-258.

Bang Jensen, B., Ersboll, A.K. and Ariel, E. (2009). Susceptibility of pike Esox lucius to a panel of Ranavirus isolates. *Diseases of Aquatic Organisms*, 83:169-179

Bang Jensen, B., Holopainen, R., Tapiovaara, H. and Ariel, E. (2011a). Susceptibility of pike-perch Sander lucioperca to a panel of ranavirus isolates. *Aquaculture* 313:24-30

Bang Jensen, B., Reschova, S., Cinkova, K., Ariel, E. and Vesely, T. (2011b). Common carp (Cyprinus carpio) and goldfish (Carassius auratus) were not susceptible to challenge with ranavirus under certain conditions. Bulletin of the European Association of Fish Pathologists, 31, 112-118.

Barray, S. and DeVauchelle, G. (1979). Etude des polypeptides de structure du virus iridescent de Chilo suppressalis (Iridovirus type 6). *Canadian Journal of Microbiology*, 25(7), 841-849.

Barray, S., and De Vauchelle, G. (1985). Protein synthesis in cells infected by Chilo iridescent virus (iridovirus, type 6). *Archives of Virology*, 86, 315-326.

Batts, W. N. and Winton, J. R (1989): Enhanced detection of infectious hematopoietic necrosis virus and other fish viruses by pretreatment of cell monolayers with poly ethytlene glycol. *Journal of Aquatic Animal Health*, 1, 284-290.

Bayley, A.E., Hill, B.J., and Feist, S.W. (2013). Susceptibility of the European common frog Rana temporaria to a panel of ranavirus isolates from fi sh and amphibian hosts. Diseases of Aquatic Organisms, 103:171–183.

Beck, B.H., Bakal, R.S., Brunner, C.J. and Grizzle, J.M. (2006). Virus distribution and signs of disease after immersion exposure to largemouth bass virus. *Journal of Aquatic Animal Health* 18:176–183.

Behncke, H., Stöhr, A. C., Heckers, K., Ball, I. and Marschang, R. E. (2013). Mass-mortality in green striped tree dragons (Japalura splendida) associated with multiple viral infections. *Vet Rec*, 173(10), 248.

Bejar, J., Borrego, J. J., and Alvarez, M. C. (1997). A continuous cell line from the cultured marine fish gilt-head seabream (*Sparus aurata L.*). *Aquaculture*,150(1), 143-153.

Bigarré, L., Cabon, J., Baud, M., Pozet, F., and Castric, J. (2008). Ranaviruses associated with high mortalities in catfish in France. *Bulletin of the European Association of Fish Pathologists*, *28*, 163-168.

Bloch, B. and Larsen, J.L. (1993). An iridovirus-like agent associated with systemic infection in cultured turbot Scophthalmus maximus fry in Denmark. *Diseases of Aquatic Organisms*, 15:235–240.

Bollinger, T.K., Mao, J., Schock, D., Brigham, R.M. and Chinchar, V.G. (1999). Pathology, isolation, and preliminary molecular characterization of a novel iridoviurs from tiger salamanders in Saskatchewan. *Journal of Wildlife Diseases*, 35:413–429.

Bondad-Reantaso, M. G., Subasinghe, R. P., Arthur, J. R., Ogawa, K., Chinabut, S., Adlard, R. and Shariff, M. (2005). Disease and health management in Asian aquaculture. *Veterinary parasitology*, 132(3), 249-272.

Bovo, G., Comuzi, M., DeMas, S., Ceschia, G., Giorgetti, G., Giacometti, P. and Cappellozza, E. (1993). Isolamento di un agente virale irido-like da pesce gatto (*Ictalurus melas)* dàllevamento. *Boll Soc Ital Patol Ittica* 11:3-10.

Brenes, R., Gray, M.J., Waltzek, T.B., Wilkes, R.P. and Miller, D.L. (2014). Transmission of ranavirus between ectothermic vertebrate hosts. *PLoS One* 9: e92476

Brown, J. D., and Sleeman, J. M. (2002). Morbidity and mortality of reptiles admitted to the Wildlife Center of Virginia, 1991 to 2000. *Journal of Wildlife Diseases*, 38(4), 699-705.

Brunner, J.L., Schock, D.M. and Collins, J.P. (2007). Transmission dynamics of the amphibian ranavirus Ambystoma tigrinum virus. *Diseases of Aquatic Organisms*, 77:87–95.

Brunner, J.L., Richards, K. and Collins, J.P. (2005). Dose and host characteristics influence virulence of ranavirus infections. *Oecologia* 144:399–406.

Brunner, J.L., Storfer, A., Gray, M.J. and Hoverman, J.T. (2015). Ranavirus ecology and evolution: from epidemiology to extinction. In: Gray M.J., Chinchar V.G. (eds) Ranaviruses: lethal pathogens of ectothermic vertebrates. *Springer,* New York

Burleson, F.G., Chambers, T. M. and Wiedbrauk, D. L. (2014). *Virology: a laboratory manual*. Elsevier

Caipang, C. M. A., Hirono, I. and Aoki, T. (2006). Immunogenicity, retention and protective effects of the protein derivatives of formalin-inactivated red seabream iridovirus (RSIV) vaccine in red seabream, Pagrus major. *Fish & shellfish immunology*, 20(4), 597-609.

Cerutti, M. and Devauchelle, G. (1982). Isolation and reconstitution of Chilo iridescent virus membrane. *Archives of virology*, 74(2-3), 145-155.

Chang, S.F., Ngoh, G.H., Kueh, L.F.S., Qin, Q.W., Chen, C.L., Lam, T.J. and Sin, Y.M., (2001). Development of a tropical marine fish cell line from Asian seabass (*Lates calcarifer*) for virus isolation. *Aquaculture* 192, 133–145.

Chang, P. S., Chen, L. J., and Wang, Y. C. (1998). The effect of ultraviolet irradiation, heat, pH, ozone, salinity and chemical disinfectants on the infectivity of white spot syndrome baculovirus. *Aquaculture*, *166*(1), 1-17.

Chao, C.B., Chen, C.Y., and Lai, Y.Y. (2004). Histological, ultrastructural, and in situ hybridization study on enlarged cells in grouper *Epinephelus* hybrids infected by grouper iridovirus in Taiwan (TGIV). *Diseases of Aquatic Organisms*, 58:127–142.

Chao, C.B., Yang, S.C., Tsai, H.Y., Chen, C.Y., Lin, C.S. and Huang, H.T. (2002). A nested PCR for the detection of grouper iridovirus in Taiwan (TGIV) in cultured hybrid grouper, giant seaperch, and largemouth bass. *Journal of Aquatic Animal Health* 14: 104–113.

Chen, Z. X., Zheng, J. C. and Jiang, Y. L. (1999). A new iridovirus isolated from soft-shelled turtle. *Virus research*, 63(1), 147-151.

Chen, Z., Gui, J., Gao, X., Pei, C., Hong, Y. and Zhang, Q. (2013). Genome architecture changes and major gene variations of Andrias davidianus ranavirus (ADRV). *Veterinary research,* 44(1), 1.

Chen, L. M., Tran, B. N., Lin, Q., Lim, T. K., Wang, F. and Hew, C. L. (2008). iTRAQ analysis of Singapore grouper iridovirus infection in a grouper embryonic cell line. *Journal of General Virology*, 89(11), 2869-2876.

Cheng, K., Jones, M.E.B., Jancovich, J.K., Burchell, J., Schrenzel, M.D., Reavill, D.R., Imai, D.I., Urban, A., Kirkendall, M., Woods, L.W., Chinchar, V.G. and Pessier, A.P. (2014). Isolation of a Bohle-like iridovirus from boreal toads housed within a cosmopolitan aquarium collection. *Diseases of Aquatic Organisms*, 111(2):139–152

Chew-Lim, M., Ngoh, G. H., Ng, M. K., Lee, J. M., Chew, P., Li, J. and Howe, J. L.C. (1994). Grouper cell line for propagating grouper viruses. *Singap. J. Prim. Ind*, *22*, 113-116.

Chia, C.B., Chun, Y.C.,Yueh, Y.L.,Chan, S.L. and Hung, T.H., (2004). Histological, ultrastructural, and in situ hybridization study on enlarged cells in grouper *Epinephelus* hybrids infected by grouper iridovirus in Taiwan (TGIV). *Diseases of Aquatic Organisms*, 58, 127-142.

Chi, S.C. (1997). The Investigation ofViral Disease Among Cultured Groupers in Southern Taiwan., pp. 59–69.

Chinchar, V.G. (2002). *Ranaviruses* (family Iridoviridae): emerging cold-blooded killers .*Archives of Virology*, 147:447-470

Chinchar, V.G. and Waltzek, T.B. (2014). *Ranaviruses*: not just for frogs. *PLoS Pathog* 10:e1003850

Chinchar, V. G. and Granoff, A. L. L. A. N. (1986). Temperature-sensitive mutants of frog virus 3: biochemical and genetic characterization. *Journal of virology*, 58(1), 192-202.

Chinchar, V. G. and Granoff, A. (1984). Isolation and characterization of a frog virus variant resistant to phosphonoacetate: genetic evidence for a virus-specific DNA polymerase. *Virology* 138:357-361.

Chinchar, V.G., Kwang, H.Y. and Jancovich, J.K. (2011). The molecular biology of frog virus 3 and other iridoviruses infecting cold-blooded vertebrates. *Viruses*;3:1959-85.

Chinchar, V. G., Hyatt, A., Miyazaki, T. and Williams, T. (2009). Family Iridoviridae: poor viral relations no longer. In Lesser Known Large dsDNA Viruses (pp. 123-170). *Springer Berlin Heidelberg.*

Fauquet, C. M., Mayo, M. A., Maniloff, J., Desselberger, U. and Ball, L. A. (Eds.). (2005). Virus taxonomy: VIIIth report of the International Committee on Taxonomy of Viruses. *Academic Press* pp. 163–175. Elsevier, London.

Choi, S.K., Kwon, S.R., Nam, Y.K., Kim, S.K. and Kim, K.H. (2006). Organ distribution of red sea bream iridovirus (RSIV) DNA in asymptomatic yearling and fingerling rock bream (Oplegnathus fasciatus) and effects of water temperature on transition of RSIV into acute phase. *Aquaculture* 256: 23–26.

Chou, H.Y., Hsu, C.C., and Peng, T.Y. (1998). Isolation and characterization of a pathogenic iridovirus from cultured grouper (*Epinephelus sp.)* in Taiwan. *Fish Pathology*. 33, 201–206

Chua, F. H. C., Ng, M. L., Ng, K. L., Loo, J. J. and Wee, J. Y. (1994). Investigation of outbreaks of a novel disease, 'Sleepy Grouper Disease', affecting the brown-spotted grouper (*Epinephelus tauvina Forskal*). *Journal of Fish Diseases*, 17, 417–427

Ch'ng, C.L. and Senoo, S. (2008). Egg and larval development of a new hybrid grouper, tiger grouper *Epinephelus fuscoguttatus* × giant grouper *E. lanceolatus*. *Aquaculture Science* 56(4): 505-515

Clem, L. W., Moewus, L., and Sigel, M. M. (1961). Studies with cells from marine fish in tissue culture. *Experimental Biology and Medicine*, 108(3), 762-766.

Cook, D.A. (2007). Web-based learning: pros, cons and controversies.*Clinical Medicine*, 7(1), 37-42.

Crane, M. S. J., Young, J., and Williams, L. M. (2005). Epizootic haematopoietic necrosis virus (EHNV): growth in fish cell lines at different temperatures. *Bulletin-European Association of Fish Pathologists*, 25(5), 228.

Cullen, B.R., and Owens, L. (2002) Experimental challenge and clinical cases of Bohle iridovirus (BIV) in native Australian anurans. *Diseases of Aquatic Organisms*, 49:83-92.

Cullen, B.R., Owens, L., and Whittington, RJ. (1995) Experimental infection of Australian anurans (Limnodynastes terraginae and Litona latopalmata) with Bohle iridovirus. *Diseases of Aquatic Organisms*, 23:83.

Cunningham, A.A., Hyatt, A.D., Russell, P., and Bennett, P.M. (2007a) 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. doi:10.1017/S0950268806007679

Cunningham, A.A., Hyatt, A.D., Russell, P., and Bennett, P.M. (2007b) Experimental transmission of a ranavirus disease of common toads (Bufo bufo) to common frogs (Rana temporaria). *Epidemiol Infect* 135:1213–1216.

Cunningham, A. A., Langton, T. E. S., Bennett, P. M., Lewin, J. F., Drury, S. E. N., Gough, R. E., and Macgregor, S. K. (1996). Pathological and microbiological findings from incidents of unusual mortality of the common frog (Rana temporaria). Philosophical Transactions of the Royal Society of London B: *Biological Sciences*, 351(1347), 1539-1557.

Daszak, P., Berger, L., Cunningham, A. A., Hyatt, A. D., Green, D. E., and Speare, R. (1999). Emerging infectious diseases and amphibian population declines. *Emerging infectious diseases,* 5(6), 735.

De, M., Ghaffar, M. A., and Das, S. K. (2014, September). Temperature effect on gastric emptying time of hybrid grouper (Epinephelus spp.). In the 2014 ukm fst postgraduate colloquium: Proceedings of the Universiti Kebangsaan Malaysia, Faculty of Science and Technology 2014 Postgraduate Colloquium (Vol. 1614, No. 1, pp. 616-618). AIP Publishing.

Delhon, G., Tulman, E. R., Afonso, C. L., Lu, Z., Becnel, J. J., Moser, B. A., and Rock, D. L. (2006). Genome of invertebrate iridescent virus type 3 (mosquito iridescent virus). *Virology journal*, 80(17), 8439-8449.

Deng, G., Li, S., Xie, J., and Bai, J. (2011). Characterization of a ranavirus isolated from cultured largemouth bass (*Micropterus salmoides*) in China. *Aquaculture* 312:198–204

Do, J.W., Cha, S.J., Kim, J.S., An, E.J., Lee, N.S., Choi, H.J., Lee, C.H., Park, M.S., Kim, J.W., Kim, Y.C., and Park, J.W. (2005). Phylogenetic analysis of the major capsid protein gene of iridovirus isolates from cultured flounders *Paralichthys olivaceus* in Korea. *Diseases of Aquatic Organisms*, 64:193–200

Docherty, D.E., Meteyer, C.U. and Wang, J. (2003). Diagnostic and molecular evaluation of three iridovirus associated salamander mortality events. *Journal of Wildlife Diseases*, 39:556–566.

Doerfler, W., Kruczek, I., Eick, D., Vardimon, L., and Kron, B. (1983, January). DNA methylation and gene activity: the adenovirus system as a model. In Cold Spring Harbor symposia on quantitative biology. *Cold Spring Harbor Laboratory Press* Vol. 47, pp. 593-603.

Dong, C.F., Weng, S.P., Shi, X.J., Xu, X.P., Shi, N. and He, J.G. (2008). Development of a mandarin fish Siniperca chuatsi fry cell line suitable for the study of infectious spleen and kidney necrosis virus (ISKNV). *Virus Research* 135: 273–281.

Dong, C.F., Xiong, X.P., Shuang, F., Weng, S.P., Zhang, J., Zhang, Y. Luo, Y.W. and He, J.G. (2011). Global landscape of structural proteins of infectious spleen and kidney necrosis virus. *Journal of Virology* 85: 2869–2877.

Drury, S. E., Gough, R. E., and Cunningham, A. A. (1995). Isolation of an iridovirus-like agent from common frogs (Rana temporaria). *Veterinary Record*, *137*(3), 72-73.

Duffus A.L.J., Waltzek T.B., Stöhr A.C., Allender M.C., Gotesman M., Whittington R.J., Hick P., Hines M.K. and Marschang R.E. (2015). Distribution and host range of ranaviruses. In: Gray MJ, Chinchar VG (eds) Ranaviruses: *lethal pathogens of ectothermic vertebrates. Springer,* New York

Eaton, H. E., Ring, B. A., and Brunetti, C. R. (2010). The genomic diversity and phylogenetic relationship in the family Iridoviridae. *Viruses,* 2(7), 1458-1475.

Eaton, H. E., Metcalf, J., and Brunetti, C. R. (2008). Expression of frog virus 3 genes is impaired in mammalian cell lines. *Virology journal*, *5*(1), 1.

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

Enriquez, R. (1993). Charakterisierung dreier Fisch-Iridovirus-Isolate vom Fluûwels (Silurus glanis), Katzenwels (Ictalurus melas) und Fluûbarsch (Perca ¯uviatilis). *Veterinary Medicine Dissertation*, Universty of Munich, Germany.

Echaubard, P., Little, K., Pauli, B., and Lesbarrères, D. (2010). Context-dependent effects of ranaviral infection on Northern leopard frog life history traits. *PLoS One,* 5(10), e13723.

Essani, K., and Granoff, A. (1989). Amphibian and piscine iridoviruses proposal for nomenclature and taxonomy based on molecular and biological properties. Intervirology, 30(4), 187-193.

Essanl, K., and Granoff, A. (1989). Amphibian and piscine iridovirus proposal for nomenclature and taxonomy based on moleular and biological properties. *intervirology* 30: 23-35.

Evelyn, T.P.T., and Traxler, G.S. (1978). Viral erythrocytic necrosis: natural occurrence in Pacific salmon and experimental transmission. *Journal of the Fisheries Board of Canada,* 35(6), 903-907.

Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U. and Ball, L.A., (2005). Virus Taxonomy. Eight Report of the International Commitee on Taxonomy of Viruses. *Elsevier Academic Presss*, USA, pp 145-161.

Fijan N., Matašin Z., Petrinec Z., Valpotic I. and Zwillingberg L.O. (1991). Isolation of an iridovirus-like agent from green frog (Rana esculenta L.). *Veterinarski Arhiv* 61:151-158.

Flegel, T. W., and MacRae, I. H. (1996). Diseases in Asian Aquaculture III, Third Symposium on Diseases in Asian Aquaculture. 29 January -2 February, Bangkok ,Fish Health Section, pp.61-66. Manila, Asian Fisheries Society.

Freshney, R.I. (2006). Culture of Animal cells, 1 ed.N.J. Hoboken, and W.S.A. John, Manual of Basic Technique, (5th Ed: 401. Glasgow G61 1BD, UK.

Freshney, R. (1987). *Culture of Animal Cells. A Manual of Basic Technique*, 2nd ed., Wiley-Liss, New York, 397 pp.

Freshney, R.I. (Ed.) (1986). *Animal cell culture: a practical approach* (Vol. 8). Oxford:: IRL press.

Fryer, J.L., and Lannan, C.N., (1994). Three decades of fish cell culture: a current listing of cell lines derived from fish. *Journal of Tissue Culture*. Methods 16, 87–94.

George, M. R., John, K. R., Mansoor, M. M., Saravanakumar, R., Sundar, P., and Pradeep, V. (2015). Isolation and characterization of a ranavirus from koi, Cyprinus carpio L., experiencing mass mortalities in India. *Journal of Fish diseases*, *38*(4), 389-403.

Getchell, R.G., Groocock, G.H., Schumacher, V.L., Grimmett, S.G., Wooster, G.A. and Bowser, P.R. (2007). Quantitative Polymerase Chain Reaction Assay for Largemouth Bass Virus. *Journal of Aquatic Animal Health* 19, 226-233.

Gibson-Kueh, S., Netto, P., Ngoh-Lim, G. H., Chang, S. F., Ho, L. L., Qin, Q. W., and Ferguson, H. W. (2003). The pathology of systemic iridoviral disease in fish. *Journal of comparative pathology*, 129(2), 111-119.

Go, J. and Whittington, R. (2006a). Experimental transmission and virulence of a megalocytivirus (Family *Iridoviridae*) of dwarf gourami (*Colisa lalia*) from Asia in Murray cod (*Maccullochella peelii peelii*) in Australia. *Aquaculture* 258: 140–149.

Go, J., Lancaster, M., Deece, K., Dhungyel, O. and Whittington, R. (2006b). The molecular epidemiology of iridovirus in Murray cod (*Maccullochella peelii peelii*) and dwarf gourami (*Colisa lalia*) from distant biogeographical regions suggests a link between trade in ornamental fish and emerging iridoviral diseases. *Molecular and Cellular Probes* 20: 212–222.

Gobbo, F., Cappellozza, E., Pastore, M. R., and Bovo, G. (2010). Susceptibility of black bullhead Ameiurus melas to a panel of ranavirus isolates. *Diseases of aquatic organisms*, 90(3), 167-174.

Goldberg, T.L. (2002). Largemouth bass virus: an emerging problem for warmwater fi sheries? In: Philipp DP, Ridgway MS (eds) Black bass: ecology, conservation and management. American Fisheries Society Symposium, Bethesda.

Goldberg T.L., Coleman D.A., Grant E.C., Inendino K.R. and Philipp D.P. (2003). Strain variation in an emerging iridovirus of warm-water fishes. *Journal of virology*, 77:8812-8818

Goorha, R. (1982). Frog virus 3 DNA replication occurs in two stages. *Journal of virology*, 43:519-528.

Goorha, R. (1981a). Frog virus 3 requires RNA polymerase II for its replication. *Journal of virology*, 37:496-499.

Goorha R. and Dixit P. (1984a). A temperature-sensitive (TS) mutant of frog virus 3 (FV3) is defective in second-stage DNA replication. *Virology* 136:186–195.

Goorha, R. and Murti, K. G. (1982). The genome of frog virus 3, an animal DNA virus, is circularly permuted and terminally redundant. *Proceedings of the National Academy of Sciences*, *79*(2), 248-252.

Goorha, R., and Granoff, A. (1979). Icosahedral cytoplasmic deoxyriboviruses. In "Comprehensive Virology" (H. Fraenkel-Conrat and R. R. Wagner, eds.), pp. 347–399. *Plenum Press*, New York.

Goorha R., Granoff A., Willis D.B. and Murti K.G. (1984b). The role of DNA methylation in virus replication: inhibition of frog virus 3 replication by 5-azacytidine. *Virology* 138:94–102.

Goorha, R., D. Willis, B., Granoff, A. and Naegele. R. F. (1981b). Characterization of a temperature-sensitive mutant of frog virus 3 defective in DNA replication. *Virology* 112:40-48.

Goorha, R.,Murti, G., Granoff, A. and Tirey, R. (1978). Macromolecular synthesis in cells infected by frog virus 3. VIII. The nucleus is a site of frog virus 3 DNA and RNA synthesis. *Virology* 84:32-50.

Gould, A. R., Hyatt, A. D., Hengstberger, S. H., Whittington, R. J., and Coupar, B. E. H. (1995). A polymerase chain reaction (PCR) to detect epizootic haematopoietic necrosis virus and bohle iridovirus. *Diseases of Aquatic Organisms*, 22:211–215.

Granoff, A. (1989). Viruses of amphibia: an historical perspective. In: Ahne W, Kurstak E (eds) Viruses of lower vertebrates. *Springer-Verlag*, Berlin, p 3–12.

Granoff, A. (1984). Frog virus 3: a DNA virus with an unusual life-style. *Progress in medical virology. Fortschritte der medizinischen Virusforschung. Progrès en virologie médicale*, 30, 187.

Granoff, A., Came, P.E. and Breeze, D.C. (1966). Viruses and renal carcinoma of Rana pipiens. I. The isolation and properties of virus from normal and tumor tissue. *Virology* 29:133-148.

Granoff, A., Came, P. E. and Rafferty, K. A. (1965). The isolation and properties of viruses from Rana pipiens: their possible relationship to the renal adenocarcinoma of the leopard frog. *Annals of the New York Academy of Sciences*, *126*(1), 237-255.

Grant, E.C., Inendino, K.R. and Love, W.J. (2005). Effects of practices related to catch-and-release angling on mortality and viral transmission in juvenile largemouth bass infected with largemouth bass virus. *Journal of Aquatic Animal Health*, 17:315–322.

Gray, M. J. and Chinchar, V. G. (2015). Introduction: History and Future of Ranaviruses. In *Ranaviruses* (pp. 1-7). Springer International Publishing.

Gray, M.J., Miller, D.L. and Hoverman, J.T. (2009). Ecology and pathology of amphibian ranaviruses. *Diseases of Aquatic Organisms* 87:243–266.

Gray, M.J., Miller, D.L., Schmutzer, A.C. and Baldwin, C.A. (2007). Frog virus 3 prevalence in tadpole populations inhabiting cattle-access and non-access wetlands in Tennessee, USA. *Diseases of Aquatic Organisms*, 77:97–103.

Greer, A.L., Berrill, M. and Wilson, P.J. (2005). Five amphibian mortality events associated with ranavirus infection in south central Ontario, Canada. *Diseases of Aquatic Organisms* 67:9-14.

Grizzle, J.M. and Brunner, C.J. (2003a). Review of largemouth bass virus. Fisheries 28:10–14.

Grizzle, J. M., Altinok, I. and Noyes, A.D. (2003b). PCR method for detection for largemouth bass virus. *Diseases of Aquatic Organisms* 54(1):29-33.

Gruia-Gray, J., and Desser, S.S. (1992) Cytopathological observations and epizootiology of frog erythrocytic virus in bullfrogs (Rana catesbeiana). *Journal of Wildlife Diseases*, 28:34–41.

Gruia-Gray, J., Petric, M. and Desser, S. (1989). Ultrastructural, biochemical and biophysical properties of an erythrocytic virus of frogs from Ontario, Canada. *Journal of Wildlife Diseases*, 25: 497–506.

Gut, J.P., Anton, M. and Bingen, A. (1981). Frog virus 3 induces a fatal hepatitis in rats. Lab Invest 45:218–228.

Haney, D.C., Hursh, D.A., Mix, M.C. and Winton, J.R.(1992). Physiological and hematological changes in chum salmon artificially infected with erythrocytic necrosis virus. *Journal of Aquatic Animal Health* 4:48–57.

Hanson, L.A., Petrie-Hanson, L., Meals, K.O., Chinchar, V.G. and Rudis, M. (2001). Persistence of largemouth bass virus infection in a northern Mississippi reservoir after a die-off. *Journal of Aquatic Animal Health* 13, 27–34.

Harikrishnan, R., Balasundaram, C., and Heo, M.S. (2010). Molecular studies, disease status and prophylactic measures in grouper aquaculture: economic importance, diseases and immunology. *Aquaculture*;309:1-14.

He, J.G., Lu, L., Deng, M., He, H.H., Weng, S.P., Wang, X.H., Zhou, S.Y., Long, Q.X., Wang, X.Z., and Chan, .SM. (2002). Sequence analysis of the complete genome of an iridovirus isolated from the tiger frog. *Virology* 292:185–197

He, J.G., Deng, M., Weng, S.P., and Li, Z. (2001). Complete genome analysis of the mandarinfish infectious spleen and kidney necrosis iridovirus. *Virology* 291:126–139

He, J. G., S. P. Wang, K. Zeng, Z. J. Huang, and S.-M. Chan.(2000). Systemic disease caused by an iridovirus-like agent in cultured mandarinfish, Siniperca chuatsi (Basilewsky), in China. *Journal of Fish Diseases*, 23:219–222.

Hedrick, R.P., and McDowell, T.S. (1995). Properties of iridoviruses from ornamental fi sh. *Vet Res* 26:423–427

Hedrick, R.P., McDowell, T.S., Ahne, W., Torhy, C., and De Kinkelin, P. (1992). Properties of three iridoviruslike agents associated with systemic infections of fi sh. *Diseases of Aquatic Organisms*, 13:203–209

Hedrick, R.P., Groff, M.J., McDowell, T. and Wingfield, W.H. (1990). An iridovirus infection of the integument of the white sturgeon *Acipenser transmontanus*. *Disease of Aquatic Organisms* 8: 39-44.

Hengstberger, S.G., Hyatt, A.D., Speare, R., and Coupar, B.E.H. (1993). Comparison of epizootic haematopoietic necrosis and Bohle iridoviruses, recently isolated Australian iridoviruses. *Diseases of Aquatic Organisms*, 15:93–107

Hetrick, F. M. and Hedrick, R. P. (1993). In: New viruses described in finfish from 1988–1992. In: *Annual Review of Fish Diseases*, M. Faisal and F. M. Hetrick, Eds, vol. 3, Pergamon Press, London, pp. 187–207.

Hoelzer, K., Shackelton, L.A. and Parrish, C.R. (2008). Presence and role of cytosine methylation in DNA viruses of animals. *Nucleic Acids Research* 36: 2825–2837.

Holopainen, R., Ohlemeyer, S., Schutze, H., Bergmann, S.M., and Tapiovaara, H. (2009). Ranavirus phylogeny and differentiation based on major capsid protein, DNA polymerase and neurofi lament triplet H1-like protein genes. *Diseases of Aquatic Organisms*, 85:81–91.

Holopainen, R., Tapiovaara, H., Honkanen, J. (2012). Expression analysis of immune response genes in fish epithelial cells following ranavirus infection. *Fish and Shellfish Immulogy* 32: 1095-1105. doi: 10.1016/j.fsi.2012.03.011.

Hossain, M., Song, J.Y., Kitamura, S.I., Jung, S.J., and Oh, M.J. (2008). Phylogenetic analysis of lymphocystis disease virus from tropical ornamental fish species based on a major capsid protein gene. *Journal of Fish Diseases* 31:473–479

Hoverman, J.T., Gray, M.J.,and Haislip, N.A. (2011). Phylogeny, life history, and ecology contribute to differences in amphibian susceptibility to ranaviruses. *Ecohealth* 8:301–319

Huang, S. M., Tu, C., Tseng, C. H., Huang, C. C., Chou, C. C., Kuo, H. C., and Chang, S. K. (2011). Genetic analysis of fish iridoviruses isolated in Taiwan during 2001–2009. *Archives of virology*, *156*(9), 1505-1515.

Huang Y, Huang X, Liu H, Gong J, Ouyang Z, Cui H, Cao J, Zhao Y, Wang X, Jiang Y, and Qin Q (2009). Complete sequence determination of a novel reptile iridovirus isolated from soft-shelled turtle and evolutionary analysis of Iridoviridae. *BMC Genomics* 10:224

Hyatt, A. D., and Whittington, R. J. (2005). Ranaviruses of fish, amphibians and reptiles: diversity and the requirement for revised taxonomy. *Diseases of Asian Aquaculture V*, 155-170.

Hyatt, A. D., Williamson, M., Coupar, B. E. H., Middleton, D., Hengstberger, S. G., Gould, A. R.,and Lee, J. (2002). First identification of a ranavirus from green pythons (Chondropython viridis). *Journal of Wildlife Diseases*, *38*(2), 239-252.

Hyatt, A. D., Gould, A. R., Zupanovic, Z., Cunningham, A. A., Hengstberger, S., Whittington, R. J., and Coupar, B. E. H. (2000). Comparative studies of piscine and amphibian iridoviruses. *Archives of virology*, *145*(2), 301-331.

Imajoh, M., Ikawa, T., and Oshima, S. I. (2007). Characterization of a new fibroblast cell line from a tail fin of red sea bream, Pagrus major, and phylogenetic relationships of a recent RSIV isolate in Japan. *Virus research*, *126*(1), 45-52.

Inoue, K., Yamano, K., Maeno, Y., Nakajima, K., Matsuoka, M., Wada, Y., and Sorimachi, M. (1992). Iridovirus infection of cultured red sea bream, Pagrus major. *Fish Pathology (Japan)*.

IUCN. 2014. IUCN Red List of Threatened Species. Version 2014. 1. IUCN 2014. IUCN Red List of Threatened Species.

Iwamoto, T., Mori, K., Arimoto, M. and Nakai, T.(1999). High permissivity of the fish cell line SSN-1 for picine noaviruse. *Diseases of Aquatic Organisms* 39: 37-47.

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

Jancovich, J. K., Davidson, E. W., Parameswaran, N., Mao, J., Chinchar, V. G., Collins, J. P., and Storfer, A. (2005). Evidence for emergence of an amphibian iridoviral disease because of human-enhanced spread. *Molecular Ecology*, *14*(1), 213-224.

Jancovich, J.K., Qin, Q., Zhang, Q.Y., and Chinchar, V.G. (2015b). Ranavirus replication: molecular, cellular, and immunological events. In: Gray MJ, Chinchar VG (eds) Ranaviruses: lethal pathogens of ectothermic vertebrates. *Springer*, New York

Jancovich, J.K., Bremont, M., Touchman, J. W., and Jacobs, B. L. (2010). Evidence for multiple recent host species shifts among the ranaviruses (family Iridoviridae). *Journal of virology*, *84*(6), 2636-2647.

Jancovich, J.K., Davidson, E. W., Seiler, A., Jacobs, B. L., and Collins, J. P. (2001). Transmission of the Ambystoma tigrinum virus to alternative hosts. *Diseases of Aquatic Organisms*, 46:159–163.

Jancovich, J.K., Davidson, E.W., Morado, J.F., Jacobs, B.L., and Collins, J.B. (1997). Isolation of a lethal virus from the endangered tiger salamander Ambystoma tigrinum stebbinsi. *Diseases of Aquatic Organisms*, 31:161–167

Jancovich, J.K., Chinchar, V.G., Hyatt, A., Miyazaki, T., Williams, T. and Zhang, Q.Y. (2012). Family *Iridoviridae*. In: Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses, ed. A.M.Q. King, M.J. Adams, E.B. Carstens and E.J. Lefkowitz, pp. 193–210. San Diego: Elsevier Academic Press.

Jancovich, J.K., Mao, J., Chinchar, V. G. , Wyatt, C., Case, S. T., Kumar, S., Valente, G., Subramanian, S., Davidson, E. W., Collins, J. P., and Jacobs, B. L. (2003). Genomic sequence of a ranavirus (family Iridoviridae) associated with salamander mortalities in North America. *Virology* 316:90–103.

Jaramillo, D., Tweedie, A., Becker, J.A., Hyatt, A., Crameri, S., and Whittington, R.J. (2012). A validated quantitative polymerase chain reaction assay for the detection of ranaviruses (Family Iridoviridae) in fi sh tissue and cell cultures, using EHNV as a model. *Aquaculture* 356:186–192

Jeong, J.B., Kim, H.Y., Kim, K.H., Chung, J.K., Komisar, J.L. and Jeong, H.D. (2006a).Molecular comparison of iridoviruses isolated from marine fish cultured in Korea and imported from China. *Aquaculture* 255: 105–116.

Jeong, J.B., Jun, L.Y., Park, K.H., Kim, K.H., Chung, J.K., Komisar, J.L., and Jeong, H.D.,(2006b). Asymptomatic iridovirus infection in various marine fishes detected by a 2- step PCR method. *Aquaculture*. 255, 30- 38.

Knowles, N.J., Hovi, T., Hyypiä, T., King, A.M.Q., Lindberg, A.M., Pallansch, M.A., and Yamashita, T. (2012). Picornaviridae. *Virus taxonomy: classification and nomenclature of viruses: ninth report of the international committee on taxonomy of viruses*, 855-880.

Jun, L.J., Jeong, J.B., Kim, J.H., Nam, J.H., Shin, K.W., Kim, J.K., Kang, J.C., and Jeong, H.D. (2009). Influence of temperature shifts on the onset and development of red sea bream iridoviral disease in rock bream Oplegnathus fasciatus. *Diseases of Aquatic Organisms* 84, 201-208.

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

Kashi Y., and King D.G. (2006). Simple sequence repeats as advantageous mutators in evolution. Trends Genet 22:253-259.

Kasornchandra, J., and Khongpradit, R. (1997). Isolation and preliminary characterization of a pathogenic iridovirus in nursing grouper, *Epinephelus malabaricus*. *Diseases in Asian Aquaculture III"(ed. by TW Flegel and IH MacRae), Manila, Philippine*, 61-66.

Kasornchandra, J., and Khongpradit, R. (1996). Isolation and preliminary characterization of a pathogenic Iridovirus-like agent in nursing grouper, Epinephilus malabaricus: Technical paper no. 5/1996.

Kattenbelt, J.A., Hyatt, A.D., and Gould, A.R. (2000). Recovery of ranavirus dsDNA from formalin-fi xed archival material. *Diseases of Aquatic Organisms*, 39:151–154

Kelly, D. C., and Tinsley, T. W. (1972). The proteins of iridescent virus types 2 and 6. *Journal of Invertebrate Pathology*, *19*(2), 273-275.

Khongpradit, R., Supamattaya, K., Kasornchandra, J., and Phromkunthong, W. (2003). Establishment of a cell line from kidney of seabass, Lates calcarifer (Bloch). *Songklanakarin Journal of Science and Technology (Thailand).*

Kim, Y. J., Jung, S. J., Choi, T. J., Kim, H. R., Rajendran, K. V., and Oh, M. J. (2002). PCR amplification and sequence analysis of irido-like virus infecting fish in Korea. *Journal of fish diseases*, *25*(2), 121-124.

Kirn, A., Bingen A, and Steffan AM (1982). Endocytic capacities of Kupffer cells isolated from the human adult liver. *Hepatology* 2:216–222.

Kirn, A., Steffan, A. M., and Bingen, A. (1980). Inhibition of erythrophagocytosis by cultured rat Kupffer cells infected with frog virus 3. *Journal of the Reticuloendothelial Society*, *28*(4), 381-388.

Kimura, T. (1983). Infectious hematopoietic necrosis of salmonid fish In "Gyobyo-gaku" (ed. by S. Egusa). Koseisha Koseikaku, Tokyo, pp. 5-13. (In Japanese.)

Kowalski, W. J., Bahnfleth, W. P., and Hernandez, M. T. (2009). A genomic model for predicting the ultraviolet susceptibility of viruses. *IUVA News*,*11*(2), 15-28.

Kurita, J., Nakajima, K., Hirono, I., and AOKI, T. (2002). Complete genome sequencing of red sea bream iridovirus (RSIV). *Fisheries science*, *68*(sup2), 1113-1115.

Lai, Y.S., John, J.A., Lin, C.H., Guo, I.C., Chen, S.C., Fang, K., Lin, C.H., and Chang, C.Y., (2003). Establishment of cell lines from a tropical grouper, Epinephelus awoara (Temminck & Schlegel), and their susceptibility to grouper irido- and nodaviruses. *Journal of Fish Diseases*, 26, 31–42.

Lai, Y.S., Chiu, H.C., Murali, S., Guo, I.C., Chen, S.C., Fang, K., and Chang, C.Y., (2001a). In vitro neutralization by monoclonal antibodies against yellow grouper nervous necrosis virus (YGNNV) and immunolocalization of virus infection in yellow grouper, Epinephelus awoara (Temminck & Schlegel). *Journal of Fish Diseases*, 24, 237–244

Lai, Y.S., Murali, S., Chiu, H.C., Ju, H.Y., Lin, Y.S., Chen, S.C., Guo, I.C., Fang, K., and Chang, C.Y., (2001b). Propagation of yellow grouper nervous necrosis virus (YGNNV) in a new nodavirus-susceptible cell line from yellow grouper, Epinephelus awoara (Temminck & Schlegel), brain tissue. *Journal of Fish Diseases*, 4, 299–309

Lai, Y.S., Murali, S., Ju, H.Y., Wu, M.F., Guo, I.C., Chen, S.C., Fang, K., and Chang, C.Y., (2000). Two iridovirus-susceptible cell lines established from kidney and liver of grouper, Epinephelus awoara (Temminck & Schlegel), and partial characterization of grouper iridovirus. *Journal of Fish Diseases*, 23, 379–388.

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

Langdon J.S. a Humphrey J.D. (1987). Epizootic haematopoietic necrosis a new viral disease in redfin perch, Perca fluviatilis L., in Australia. *Journal of Fish Diseases* 10, 289–298.

Langdon, J. S., Humphrey, J. D., and Williams, L. M. (1988a). Editorial responsibility: Managing Editor Outbreaks of EHNV-11ke lndovlrus in cultured rainbow trout, Salmo gairdneri Richardson, In Austraha. *Journal of Fish Diseases*. 11. 93-96

Langdon J.S., Humphrey J.D. and Williams L.M. (1988b). Outbreaks of an EHNV-like iridovirus in cultured rainbow trout, Salmo gairdneri Richardson, in Australia. *Journal of Fish Diseases* 11, 93–96.

Langdon, J. S., Humphrey, J. D., and Copland, J. (1986a). The disease status of Australian salmonids: viruses and viral diseases. *Journal of Fish Diseases* 9:129–135

Langdon, J. S., Humphrey, J. D.,and Williams, L. M. (1986b). First virus isolation from Australian fi sh: an iridovirus-like pathogen from redfi n perch, Perca fl uviatilis L. *Journal of Fish Diseases* 9:263–268

Leimbach, S., Schütze, H., and Bergmann, S. M. (2014). Susceptibility of European sheatfish Silurus glanis to a panel of ranaviruses. *Journal of Applied Ichthyology*, *30*(1), 93-101.

Leong, J. C., J. L. Fendrick, S. Youngman and A. Lee (1981). Effect of polybrene on the infectivity of infec tious haematopoietic necrosis virus in tissue culture. *Journal of Fish Diseases*, 4, 335-344.

Lesbarrères, D., Balseiro, A., Brunner, J., Chinchar, V. G., Duffus, A., Kerby, J., and Gray, M. J. (2012). Ranavirus: past, present and future.*Biology letters*, *8*(4), 481-483.

Li, W., Zhang, X., Weng, S., Zhao, G., He, J., and Dong, C. (2014). Virion-associated viral proteins of a Chinese giant salamander (Andrias davidianus) iridovirus (genus Ranavirus) and functional study of the major capsid protein (MCP). *Veterinary microbiology*, *172*(1), 129-139.

Lua, D.T., Yasuike, M., Hirono, I. and Aoki, T. (2005). Transcription program of red sea bream iridovirus as revealed by DNA microarrays. *Journal of Virology* 79: 15151–15164.

Lü, L., Zhou, S. Y., Chen, C., Weng, S. P., Chan, S. M., and He, J. G. (2005). Complete genome sequence analysis of an iridovirus isolated from the orange-spotted grouper, Epinephelus coioides. *Virology*, *339*(1), 81-100.

MacMillan, J. R., and Mulcahy, D. (1979). Artificial transmission to and susceptibility of Puget Sound fish to viral erythrocytic necrosis (VEN)[British Columbia]. *Journal of the Fisheries Research Board of Canada (Canada)*.

Mahardika, K., Zafran, Yamamoto, A. and Miyazaki, T. (2004). Susceptibility of juvenile humpback grouper *Cromileptes altivelis* to grouper sleepy disease iridovirus (GSDIV). *Diseases of Aquatic Organisms* 59: 1–9.

Mao JH, Wang J, and Chinchar GD (1999a). Molecular characterization of a ranavirus isolated from largemouth bass Micropterus salmoides . *Diseases of Aquatic Organisms* 37:107–114

Mao J, Hedrick RP, and Chinchar VG (1997). Molecular characterization, sequence analysis, and taxonomic position of newly isolated fi sh iridoviruses. *Virology* 229:212–220

Mao, J., Green, D. E., Fellers, G., and Chinchar, V. G. (1999b). Molecular characterization of iridoviruses isolated from sympatric amphibians and fish. *Virus research*, *63*(1), 45-52.

Marschang, R.E., Braun, S., and Becher, P. (2005). Isolation of a ranavirus from a gecko (Uroplatus fimbriatus). *Journal of Zoo and Wildlife Medicine*, *36*(2), 295-300.

Marschang, R.E., Becher, P., Posthaus, H., Wild, P., Thiel, H. J., Müller-Doblies, U., and Bacciarini, L. N. (1999). Isolation and characterization of an iridovirus from Hermann's tortoises (Testudo hermanni). *Archives of virology*, *144*(10), 1909-1922.

Marsh, I.B., Whittington, R.J., Hyatt, A. D., &and Chisholm, O. (2002). Rapid differentiation of Australian, European and American ranaviruses based on variation in major capsid protein gene sequence. *Molecular and Cellular Probes*, *16*(2), 137-151.

Martin, J.P., Aubertin, A.M., Tondre, L., and Kirn, A. (1984). Fate of frog virus 3 DNA replicated in the nucleus of arginine-deprived CHO cells. *Journal of general virology*, *65*(4), 721-732.

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

McCallum, H., Barlow, N., and Hone, J. (2001). How should pathogen transmission be modelled? Trends Ecol Evol 16:295–300

McClenahan, S. D., Beck, B. H., and Grizzle, J. M. (2005). Evaluation of cell culture methods for detection of largemouth bass virus. *Journal of Aquatic Animal Health*, *17*(4), 365-372.

McGrogan, D. G., Ostland, V. E., Byrne, P. J. and Ferguson, H. W. (1998). Systemic disease involving an iridoviruslike agent in cultured tilapia,Oreochromis niloticus L.—a case report. *Journal of Fish Diseases*, 21, 149–152.

Meng, Y., Ma, J., Jiang, N., Zeng, L. B., and Xiao, H. B. (2014). Pathological and microbiological findings from mortality of the Chinese giant salamander (Andrias davidianus). Archives of virology, 159(6), 1403-1412.

Medzhitov, R., Schneider, D. S., and Soares, M. P. (2012). Disease tolerance as a defense strategy. *Science*, *335*(6071), 936-941.

Meyers, T. R. (Ed.) (2000). *Fish pathology section laboratory manual*. Alaska Department of Fish and Game, Commercial Fisheries Division.

Meyer, F.P. (1991). Aquaculture disease and health management. *Journal of Animal Science* 69: 4201–4208.

Meyers, T.R., Hauck, A.K., Blankenbeckler, W.D., and Minicucci, T. (1986). First report of viral erythrocytic necrosis in Alaska, USA, associated with epizootic mortality in Pacific herring, Clupea harengus pallasi (Valenciennes). *Journal of Fish Diseases* 9:479–491

Miller, D.L., Gray, M.J., and Strofer, A. (2011). Ecopathology of ranaviruses infecting amhibians. *Viruses* 3:2351–2373.

Miller, D.L., Pessier, A.P., Hick, P., and Whittington, R.J. (2015). Comparative pathology of ranaviruses and diagnostic techniques. In: Gray MJ, Chinchar VG (eds) Ranaviruses: lethal pathogens of ectothermic vertebrates. *Springer*, New York.

Miller, D.L., Rajeev, S., Gray, M.J., and Baldwin, C. (2007). Frog virus 3 infection, cultured American bullfrogs. *Emerg Infect Dis* 13:342–343.

Miyata, M., Matsuno, K., Jung, S.J., Danayadol, Y., and Miyazaki, T. (1997). Genetic similarity of iridoviruses from Japan and Thailand *Journal of Fish Diseases*. 20:127-134

Moody, N. J. G., and Owens, L. (1994). Experimental demonstration of the pathogenicity of a frog virus, Bohle iridovirus, for a fish species, barramundi Lates calcarifer. *Diseases of Aquatic Organisms*, *18*(2), 95-102.

Moya, A., Holmes, E. C., and González-Candelas, F. (2004). The population genetics and evolutionary epidemiology of RNA viruses. *Nature Reviews Microbiology*, *2*(4), 279-288.

Murali, S., Wu, M. F., Guo, I. C., Chen, S. C., Yang, H. W., and Chang, C. Y. (2002). Molecular characterization and pathogenicity of a grouper iridovirus (GIV) isolated from yellow grouper, Epinephelus awoara (Temminck & Schlegel). *Journal of fish diseases*, *25*(2), 91-100.

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

Handayani, C. R., and Pratiwi, R. (2015). Cloning and Sequence Analysis of Capsid Protein Gene of Iridovirus Indonesian Isolates. *Indonesian Journal of Biotechnology*, *14*(1).

Nagasawa, K., and Cruz-Lacierda, E. R. (2004). *Diseases of cultured groupers*. Iloilo, Philippines: Southeast Asian Fisheries Development Center, Aquaculture Department.

Nakajima, K. and Sorimachi, M. (1994). Biological and physico-chemical properties of the iridovirus isolated from the cultured red sea bream, Pagrus major. *Fish Pathology*, 29, 29–33.

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

Nakajima, K., Ito, T., Kurita, J., Kawakami, H., Itano, T., Fukuda, Y. Aoki, Y., Tooriyama, T. and Manabe, S. (2002). Effectiveness of a vaccine against red sea bream iridoviral disease in various cultured marine fish under laboratory conditions. *Fish Pathology* 37: 90–91

Neukirch, M. and P. Kruse (1993): Enhancement of the in vitro infectivity of V834 in the presence of trypsin. *Journal of Fish Diseases* 16, 155-159.

Nicholson, B. L., Danner, D. J., and Wu, J. L. (1987). Three new continuous cell lines from marine fishes of Asia. *In vitro cellular & developmental biology*, *23*(3), 199-204.

Ogawa, M., Ahne, W., Fischer-Scherl, T., Hoffmann, R. W., and Schlotfeldt, H. J. (1990). Pathomorphological alterations in sheatfish fry Silurus glanis experimentally infected with iridovirus-like agent. *Diseases of aquatic organisms*, *9*(3), 187-191.

Oh, S. Y., Oh, M. J., and Nishizawa, T. (2014). Potential for a live red seabream iridovirus (RSIV) vaccine in rock bream Oplegnathus fasciatus at a low rearing temperature. *Vaccine*, *32*(3), 363-368.

Oh, M.J., Kitamura, S.I., Kim, W.S., Park, M.K., Jung, S.J., Miyadai, T., and Ohtani, M., (2006). Susceptibility of marine fish species to a megalocytivirus, turbot iridovirus, isolated from turbot, Psetta maximus (L.). *Journal of Fish Diseases* 29, 415–421.

OIE (2011). Diseases listed by the OIE. In: Aquatic Animal Health Code, Chapter 1.3. *World Organisation for Animal Health*

OIE (2012). Chapter 2.3.1 Epizootic Haematopoietic necrosis. In: Manual of diagnostic tests for aquatic animals (*World Organisation for Animal Health*).

Orange, N., and Devauchelle, G. (1987). Lipophilic polypeptides of Chilo iridescent virus (CIV, type 6) membrane. *FEMS microbiology letters*, 48(1-2), 59-64.

Oshima, S. I., Hata, J. I., Segawa, C., Hirasawa, N., and Yamashita, S. (1996). A method for direct DNA amplification of uncharacterized DNA viruses and for development of a viral polymerase chain reaction assay: application to the red sea bream iridovirus. *Analytical biochemistry*, *242*(1), 15-19.

Ott, T. (2004). Tissue culture of fish cell lines. NWFHS Laboratory procedures manual, 1-16.

Pallister, J., Gould, A., Harrison, D., Hyatt, A., Jancovich, J., and Heine, H. (2007) Development of real-time PCR assays for the detection and differentiation of Australian and European ranaviruses. *Journal of Fish Diseases* 30:427–438

Paperna, I, M. Vilenkin, and A. P. de Matos. (2001). Iridovirus infections in farm-reared tropical ornamental fish. *Diseases of Aquatic Organisms* 48:17–25.

Pasmans, F., Blahak, S., Martel, A., Pantchev, N., and Zwart, P. (2008). Ranavirus-associated mass mortality in imported red tailed knobby newts (Tylototriton kweichowensis): a case report. *The Veterinary Journal*, *176*(2), 257-259.

Picco, A.M., Karam, A.P., and Collins, J.P. (2010). Pathogen host switching in commercial trade with management recommendations. *Ecohealth* 7:252–256

Plumb, J.A., and Hanson, L.A. (2011). Health maintenance and principal microbial diseases of cultured fi shes. *Wiley*, Hoboken

Plumb, J. A., and Zilberg, D. (1999). Survival of largemouth bass iridovirus in frozen fish. *Journal of Aquatic Animal Health*, *11*(1), 94-96.

Plumb, J.A., and Zilberg, D. (1999b). The lethal dose of largemouth bass virus in juvenile largemouth bass and the comparative susceptibility of striped bass. *Journal of Aquatic Animal Health* 11:246–252

Plumb, J.A., Grizzle, J.M., and Young, H.E. (1996). An iridovirus isolated from wild largemouth bass. *Journal of Aquatic Animal Health* 8:265–270

Plumb, J.A., Noyes, A.D., Graziano, S., Wang, J., Mao, J.H. and Chinchar, V.G. (1999). Isolation and identification of viruses from adult largemouth bass during a 1997–1998 survey in the southeastern United States. *Journal of Aquatic Animal Health* 11, 391–399.

Pozet, F., Morand, M., Moussa, A., Torhy, C., and De Kinkelin, P. (1992). Isolation and preliminary characterization of a pathogenic icosahedral deoxyribovirus from the catfish Ictalurus melas. Diseases of Aquatic Organisms, 14(1), 35-42.

Prasankok, P. O. N. G. P. U. N., Chutmongkonkul, M. A. L. I. N. E. E., and Kanchanakhan, S. O. M. K. I. A. T. (2005). Characterisation of iridovirus isolated from diseased marbled sleepy goby, Oxyeleotris marmoratus. Diseases in Asian Aquaculture V. Asian Fisheries Society, Manila.

Qu, X. X., Hao, P., Song, X. J., Jiang, S. M., Liu, Y. X., Wang, P. G., and Zheng, A. H. (2005). Identification of two critical amino acid residues of the severe acute respiratory syndrome coronavirus spike protein for its variation in zoonotic tropism transition via a double substitution strategy. *Journal of Biological Chemistry*, *280*(33), 29588-29595.

Qin, Q.W., Chang, S.F., and Ngoh-lim, G.H. (2003). Characterization of a novel ranavirus isolated from grouper Epinephelus tauvina . *Diseases of Aquatic Organisms* 53:1–9

Qin, Q. W., Lam, T. J., Sin, Y. M., Shen, H., Chang, S. F., Ngoh, G. H. and Chen, C. L. (2001). Electron microscopic observations of a marine fish iridovirus isolated from brown-spotted grouper, Epinephelus tauvina. *Journal of Virological Methods*, 98, 17–24.

Raghow, R., and Granoff. A. (1980). Macromolecular synthesis in cells infected with frog virus 3. XIV. Characterization of the methylated nucleotide sequences in viral messenger RNA. *Virology* 107:283-294.

Razin, A., and Riggs. A. D. (1980). DNA methylation and gene function. *Science* 210:604-610.

Reddacliff, L.A. and Whittington, R.J. (1996). Pathology of epizootic haematopoietic necrosis virus (EHNV) infection in rainbow trout (Oncorhynchus mykiss Walbaum) and redfin perch (Perca fluviatilis L). *Journal of Comparative Pathology* 115, 103–115.

Reed, L. J., and Muench, H. (1938). A simple method of estimating fifty per cent endpoints. *American journal of epidemiology* 27(3), 493-497.

Rodgers, H. D., Kobs, M., Macartney, A. and Frerichs, G. N. (1997). Systemic iridovirus infection in freshwater angelfish, Pterophyllum scalare Lichtenstein. *Journal of Fish Diseases* 20, 69–72.

Robert, J., Morales, H., Buck, W., Cohen, N., Marr, S. and Gantress, J. (2005). Adaptive immunity and histopathology in frog virus 3-infected Xenopus. *Virology* 332, 667–675.

Rojas, S., Richards, K., Jancovich, J.K., and Davidson, E.W. (2005). Influence of temperature on Ranavirus infection in larval salamanders Ambystoma tigrinum. *Diseases of Aquatic Organisms* 63:95-100

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). *Molecular cloning* (Vol. 2, pp. 14-9). New York: *Cold spring harbor laboratory press*.

Sample, R. (2010). Elucidation of Frog Virus 3 gene function and pathways of virion formation. Ph.D. dissertation, University of Mississippi Medical Center, Jackson, MS

Schetter, C., Grunemann, B., Holker, I. and Doerfler, W. (1993). Patterns of frog virus 3 DNA methylation and DNA methyltransferase activity in nuclei of infected cells. *Journal of Virology* 67: 6973–6978.

Schramm, J.r., H. L., and Davis, J. G. (2006). Survival of largemouth bass from populations infected with largemouth bass virus and subjected to simulated tournament conditions. *North American journal of fisheries management*, *26*(4), 826-832.

Senoo, S. (2006). Hybrid production between tiger grouper Epinephelus fuscoguttatus × giant grouper Epinephelus lanceolatus (fish culture in Southeast Asia 64). *Aquanet Magazine* 12: 58-63.

Smith, K. M. and Xeros, N. (1954). An unusual virus disease of a dipterous larva. *Nature* 173, 866–867

Song, W.J., Qin, Q.W., Qiu, J., Huang, C.H., Wang, F., and Hew, C.L. (2004). Functional genomics analysis of Singapore grouper iridovirus: complete sequence determination and proteomic analysis. J Virol 78:12576–12590.

Speare, R., and Smith, J. R. (1992). An iridovirus-like agent isolated from the ornate burrowing frog Limnodynastes ornatus in northern Australia. *Diseases of Aquatic Organisms*, *14*, 51-51.

Sudthongkong, C., Miyata, M. and Miyazaki, T. (2002a). Iridovirus disease in two ornamental tropical freshwater fishes: African lampeye and dwarf gourami. *Disease of Aquatic Organisms* 48: 163–173.

Subramaniam, K. (1999). grouper aquaculture development in Malaysia.

Sudthongkong, C., Miyata, M. and Miyazaki, T. (2002b). Viral DNA sequences of genes encoding the ATPase and the major capsid protein of tropical iridovirus isolates which are pathogenic to fishes in Japan, South China Sea and Southeast Asian countries. *Archives of Virology* 147: 2089–2109

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28, 2731–2739.

Tamaru, Y., Ohtsuka, M., Kato, K., Manabe, S., Kuroda, K., Sanada, M., and Ueda, M. (2006). Application of the arming system for the expression of the 380R antigen from red sea bream iridovirus (RSIV) on the surface of yeast cells: a first step for the development of an oral vaccine. *Biotechnology progress*, *22*(4), 949-953.

Tan WGH, Barkman TJ, Chinchar VG, Essani K (2004) Comparative genomic analyses of frog virus 3, type species of the genus Ranavirus (family Iridoviridae). *Virology* 323:70–84.

Tapiovaara, H., Olesen, N. J., Lindén, J., Rimaila-Pärnänen, E., and von Bonsdorff, C. H. (1998). Isolation of an iridovirus from pike-perch Stizostedion lucioperca. *Diseases of aquatic organisms*, *32*(3), 185-193.

Thompson, J.P., Granoff, A. and Willis, D.B. (1987). Infection with frog virus 3 allows transcription of DNA methylated at cytosine but not adenine residues. *Virology* 160: 275–277.

Tidona, C.A., Schnitzler, P., Kehm, R. and Darai, G. (1998). Is the Major capsid protein of iridoviruses a suitable target for the study of viral evolution? *Virus Genes* 16: 59–66.

Trust, T. (1986). Pathogenesis of infectious diseases of fish. *Annual Reviews in Microbiology* 40: 479–502.

Tsai, C. T., Ting, J. W., Wu, M. H., Wu, M. F., Guo, C., and Chang, C. Y. (2005). Complete genome sequence of the grouper iridovirus and comparison of genomic organization with those of other iridoviruses. *Journal of virology*, *79*(4), 2010-2023.

Ultsch, G. R. (2006). The ecology of overwintering among turtles: where turtles overwinter and its consequences. *Biological reviews*, *81*(03), 339-367.

Vesely, T., K. Cinkova, S. Reschova, F. Gobbo, E. Ariel, M. Vicenova, D. Pokorova, P. Kulich, and G. Bovo. (2011). Investigation of ornamental fish entering the EU for the presence of ranaviruses. *Journal of Fish Diseases* 34:159–166.

Waltzek, T.B., Miller, D.L., Gray, M.J., Drecktrah, B., Briggler, J.T., MacConnell. B., Hudson, C., Hopper, L., Friary, J., Yun, S.C., Malm, K.V., Weber, E.S., and Hedrick, R.P. (2014). Expansion of the host range of frog virus 3 into hatchery-reared pallid sturgeon Scaphirhynchus albus. *Diseases of Aquatic Organisms*111:219–227. doi: 10.3354/dao02761

wamoto, T., Mori, K., Arimoto Iand, M., and Nakai, T. (1999). High permissivity of the fish cell line SSN-1 for piscine nodaviruses. *Diseases of Aquatic Organisms* 39,37-47.

Wang, Y.Q., Lu, L., Weng, S.P., Huang, J.N., Chan, S.M., and He, J.G. (2007). Molecular epidemiology and phylogenetic analysis of a marine fish infectious spleen and kidney necrosis virus-like (ISKNVlike) virus. *Archives of Virology* 152:763–773

Wang, J.W., Deng, R.Q., Wang, X.Z., Huang, Y.S., Xing, K., Feng, J.H., He, J.G. and Long, Q.X. (2003). Cladistic analysis of iridoviruses based on protein and DNA sequences. *Archives of Virology* 148: 2181–2194.

Weir, R. P., Moody, N. J. G., Hyatt, A. D., Crameri, S., Voysey, R., Pallister, J., and Jerrett, I. V. (2012). Isolation and characterisation of a novel Bohle-like virus from two frog species in the Darwin rural area, Australia. *Diseases of aquatic organisms*, *99*(3), 169-177.

Whittington, R. J., and Reddacliff, G. L. (1995). Influence of environmental temperature on experimental infection of redfin perch (Perca fluviatilis) and rainbow trout (Oncorhynchus mykiss) with epizootic haematopoietic necrosis virus, an Australian iridovirus. *Australian Veterinary Journal*, *72*(11), 421-424.

Whittington, R.J. and Chong, R. (2007). Global trade in ornamental fish from an Australian perspective: the case for revised import risk analysis and management strategies. *Preventive Veterinary Medicine* 81: 92–116

Whittington RJ, Becker JA, and Dennis MM (2010). Iridovirus infections in fi nfi sh—critical review with emphasis on ranaviruses. *Journal of Fish Diseases* 33:95–122.

Whittington, R. J., Philbey, A., Reddacliff, G. L. and MacGown, A. R. (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. *Journal of Fish Diseases*, 17, 205–218.

Williams, T. (1996). The iridoviruses. *Advances in Virus Research* 46: 347–412

Williams, T., Barbosa-Solomieu, V. and Chinchar, V. G. (2005). A decade of advances in iridovirus research. *Advances in Virus Research* 65, 173–248.

Williams, T., Chinchar, G., Darai, G., Hyatt, A., Kalmakoff, J., and Seligg, V. (2000). Family Iridoviridae. InVirus Taxonomy - 7th report of the international committee on taxonomy of viruses; Regenmortel, M.H.V., Ed.; *Academic Press*: New York, U.S.A, pp. 167–182.

Willis, D. B., and A. Granoff. (1978). Macromolecular synthesis in cells infected with frog virus 3. IX. Two temporal classes of early RNA. *Virology* 68:443-453.

Willis, D. B., and A. Granoff. (1980). Frog virus 3 DNA is heavily methylated at CpG sequences. *Virology* 107:250-257.

Willis, D.B.; Granoff, A. (1985). Transactivation of an immediate-early frog virus 3 promoter by a virion protein. *Journal of Virology* 56, 495-501.

Willis DB, Goorha R, and Granoff A (1984). DNA methyltransferase induced by frog virus 3. *Journal of Virology* 49:86–91

Willis, D. B., Goorha, R., and Chinchar, V. G. (1985). Macromolecular synthesis in cells infected by frog virus 3. In *Iridoviridae* (pp. 77-106). Springer Berlin Heidelberg.

Wolf, K. (1988). Lymphocystis disease. In: Fish Viruses and Fish Viral Disease, K. Wolf, Ed., *Cornell University Press*, New York, pp. 268–291.

Wolf, K.M., and Quimby, M., (1966). Lymphocystis virus: isolation and propagation in centrachid fish cell lines. *Science* 151, 1004–1005

Wolf, K., Bullock, G. L., Dunbar, C. E., and Quimby, M. C. (1968). Tadpole edema virus: a viscerotropic pathogen for anuran amphibians. *The Journal of infectious diseases*, 253-262.

Woodhams, D.C., Alford, R.A., and Marantelli, G. (2003). Emerging disease of amphibians cured by elevated body temperature. *Diseases of Aquatic Organisms*55:65–67

Woodland, J. E., Noyes, A. D., and Grizzle, J. M. (2002). A survey to detect largemouth bass virus among fish from hatcheries in the southeastern USA. *Transactions of the American Fisheries Society*, *131*(2), 308-311.

Xeros, N. (1954). A second virus disease of the leather jacket, Tipula paludosa. *Nature* 174: 562–563.

Xu, X., Huang, L., Weng, S., Wang, J., Lin, T., Tang, J. Li, Z., Lu, Q., Xia, Q., Yu, X. and He, J. (2010). Tetraodon nigroviridis as a nonlethal model of infectious spleen and kidney necrosis virus (ISKNV) infection. *Virology* 406: 167–175.

Yuan, T.T.T., Lin, M.H., Chen, D.S. and Shih, C. (1998). A Defective Interference-Like Phenomenon of Human Hepatitis B Virus in Chronic Carriers. *Journal of Virology* 72: 578–584.

Zilberg, D., Grizzle, J.M., and Plumb, J.A. (2000). Preliminary description of lesions in juvenile largemouth bass injected with largemouth bass virus. *Diseases of Aquatic Organisms* 39:143–146.

Zhang, M., Hu, Y. H., Xiao, Z. Z., Sun, Y., and Sun, L. (2012). Construction and analysis of experimental DNA vaccines against megalocytivirus. *Fish & shellfish immunology 33*(5), 1192-1198.

Zhang, Q. Y., Xiao, F., Xie, J., Li, Z. Q., and Gui, J. F. (2004). Complete genome sequence of lymphocystis disease virus isolated from China. *Journal of Virology 78*(13), 6982-6994.

Zhang, Q.Y., Xiao, F., Li, Z.Q., Gui, J.F., Mao, J., and Chinchar, V.G. (2001). Characterization of an iridovirus from the cultured pig frog Rana grylio with lethal syndrome. *Diseases of Aquatic Organisms* 48:27-36

Zupanovic, Z., Musso, C., Lopez, G., Louriero, C.L., Hyatt, A.D., Hengstberger, S., and Robinson, A.J. (1998). Isolation and characterization of iridoviruses from the giant toad Bufo marinus in Venezuela. *Diseases of Aquatic Organisms* 33:1-9.