Frontiers in Environmental Microbiology
2023; 9(2): 18-23
http://www.sciencepublishinggroup.com/j/fem
doi: 10.11648/j.fem.20230902.11
ISSN: 2469-7869 (Print); ISSN: 2469-8067 (Online)

Lymphocystis Disease Virus in Largemouth Bass - A Case Report

Suja Aarattuthodi

Thad Cochran National Warmwater Aquaculture Center, Delta Research and Extension Center, Mississippi Agriculture and Forestry Experiment Station, Mississippi State University, Stoneville, USA

Email address:
bsa122@msstate.edu

To cite this article:
Suja Aarattuthodi. Lymphocystis Disease Virus in Largemouth Bass - A Case Report. *Frontiers in Environmental Microbiology*. Vol. 9, No. 2, 2023, pp. 18-23. doi: 10.11648/j.fem.20230902.11

Received: May 15, 2023; **Accepted**: June 1, 2023; **Published**: June 10, 2023

Abstract: Lymphocystis disease caused by the lymphocystis disease virus (LCDV) infects numerous fish species including wild and farmed fish from several families. The LCDV is a DNA virus belonging to the family Iridoviridae. The virus usually causes benign, chronic infection characterized by macroscopic nodules located on the body, fins, and gills of fish, although other tissues may be infected occasionally. The disease normally does not cause significant mortalities, but massive infections leading to substantial mortalities have been reported in some aquacultured species such as sea bream (*Sparus aurata*), Olive flounder (*Paralichthys olivaceus*), Japanese seabass (*Lateolabrax japonicus*), and rockfish (*Sebastes schlegeli*). In this study, nodules from the fins of a largemouth bass (*Micropterus salmoides*) were subjected to pathological examination. Microscopic analysis of the wet mount and the histological analysis revealed the pathological alterations caused by the lymphocystis disease virus. Histopathological analysis indicated that the nodules contained encapsulated hypertrophic cells typical of the viral infection. The inoculum from the fin nodules produced cytopathic effects (CPEs) on Bluegill Fry (BF-2) cells, while no CPEs were observed in the Epithelioma Papulosum Cyprini (EPC) and Fathead Minnow (FHM) cell lines. These findings suggest that the causative agent of the fin infection in the largemouth bass is lymphocystis disease virus.

Keywords: Lymphocystis Disease Virus, Largemouth Bass, Nodules, Histopathology, Cytopathic Effects

1. Introduction

Lymphocystis is an infectious viral disease reported from several (>140) freshwater, estuarine, and marine fish species worldwide [1-10]. The etiologic agent is lymphocystis disease virus (LCDV) belonging to the family Iridoviridae [11-14]. Lymphocystis is reported in both wild and farmed fish including food fish and ornamental fish [15-20]. The virion is icosahedral (120- 300 nm) with linear single- or double-stranded DNA [21-23]. Several LCDV strains have been reported [24-26].

Lymphocystis disease virus mainly affects advanced families of fishes including Cichlidae, Centrarchidae, Gobiidae, Pomacentridae, Sciaenidae, Serranidae, etc. [6, 27]. The virus causes benign and chronic infection characterized by macroscopic nodules located mostly on the body, fins, and gills [28], but other tissues may be affected [29]. The nodules can appear white, gray, pink or red depending on the degree of vascularity. In early stages of infection, the relatively small nodules may be confused with parasites or skin neoplasia. The infected lymphocysts possess a distinct hyaline-like capsule, an enlarged nucleus, and segmented, basophilic cytoplasmic inclusions that contain developing virions [30-32]. The infected cells are 50,000–100,000 times larger than normal cells which subsequently burst and shed virus into the environment [32].

Transmission of the virus is horizontal, most often from infected fish to fish that has damaged or abraded skin surface [1]. The onset and duration of infection is temperature dependent [1]. Disease progression is highly variable with fish in colder temperatures having lesions persisting longer, while fish in warmer temperatures may resolve the lesions within a few weeks. Stress from spawning, crowding, handling, parasites, etc. can trigger disease outbreaks and are exacerbated by stressors such as poor water quality, poor diet or suboptimal temperatures [33-35].

The disease normally does not cause significant mortalities. However, heavy infections may impair fish respiration and/or

feeding. LCDV has been associated with significant mortality in farmed fish [36-37]. Based on the reports from disease outbreaks, young fish appear to be more susceptible. In Korea and Japan, Olive flounder (*Paralichthys olivaceus*), Japanese seabass (*Lateolabrax japonicus*), and rockfish (*Sebastes schlegeli*) aquaculture industries are significantly affected by LCD [34, 38]. Massive infections causing significant mortalities were recorded in juvenile sea bream (*Sparus aurata*) in grow-out facilities [39]. Mortalities (~45%) in juvenile fish are reported, especially when secondary bacterial infections are involved. Since effective treatments are limited, disease prevention relies on good husbandry practices.

Lymphocystis is diagnosed by identification of the characteristic cytomegalic fibroblasts by wet mount preparations or histology of skin or gill [6, 32], virus isolation using susceptible cell lines, and or by PCR. The Iridoviridae family has conserved regions within the major capsid protein (MCP) gene and DNA polymerase gene making those ideal molecular markers for the identification of the virus [40-43].

Lymphocystis disease seems to be ubiquitous in the aquatic environment and has been reported from different habitats. The global distribution and wide host range of LCDV point to the economic impacts of these pathogens [44-45]. The viral infection can cause significant economic losses especially in the ornamental trade as affected fish appear unsightly and may cause market rejection. In the current study, the presence of LCDV in an infected largemouth bass (*Micropterus salmoides*) was investigated.

2. Materials and Methods

2.1. Infected Fish

The infected largemouth bass (body weight ~50 g) was from a large aquarium tank maintained at the Thad Cochran National Warmwater Aquaculture Center, Stoneville, MS in the fall of 2021. Clusters of nodules located on the pectoral fins of the fish (Figure 1) were observed. Fish displaying external lesions was collected and the nodules were dissected out aseptically from the fins for pathological analyses.

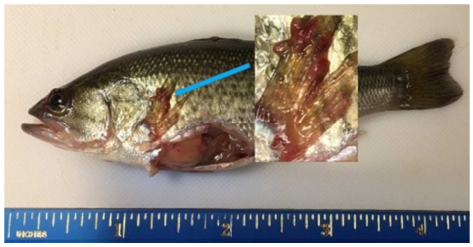

Figure 1. *Lymphocystis Disease in a Young Largemouth Bass (Micropterus Salmoides). Clusters of Nodules (Blue Arrow) Characteristic of the Lymphocystis Disease Virus (LCDV) Infection are Visible on the Pectoral Fin of the Fish.*

2.2. Wet Mount of the Largemouth Bass Fin Nodules

Fin nodules from the infected largemouth bass were aseptically dissected out and used for a wet mount preparation. The wet mounts were viewed under a light microscope.

2.3. Histopathological Analysis

For histopathological analysis, the collected nodules from the fin of the infected fish were fixed in 10% neutral-buffered formalin (NBF), processed, and embedded in paraffin [14]. The sections were stained with hematoxylin and eosin (H&E) and observed under a microscope.

2.4. Use of Cell Lines for the Isolation of Virus from the Infected Fish Tissues

The virus inoculum was prepared by homogenizing the samples in 1X Hank's balanced salt solution (HBSS) using a tissuelyser (Qiagen, Germantown, MD), which was then centrifuged at 2000 xg at 4°C for 10 min. Cell lines used were BF-2 (Bluegill Fry), EPC (Epithelioma Papulosum Cyprini), and FHM (Fathead Minnow), all from ATCC (American Type Culture Collection). The cell culture media consisted of Leibovitz medium (L-15) supplemented with 10% fetal bovine serum (FBS), antibiotics, and fungicide. Cells were grown on 24-well plates until confluence and inoculated with 100 uL of virus suspension. The virus suspension was prepared from tissuelysed fin nodules, which was filtered and inoculated onto healthy confluent BF-2, FHM, and EPC cell monolayers, and incubated at 25°C. Triplicates and control cells without virus inoculum were maintained. The cell monolayers were routinely examined for cytopathic effects (CPEs) using an inverted microscope (Zeiss Primovert, NY, USA).

2.5. Molecular Analyses

Genomic DNA used as template for PCR was extracted from the BF-2 cells showing CPEs. Infected cells along with the media were centrifuged at 5000 xg for 10 min and used for DNA extraction using the Blood and Tissue Kit (Qiagen) according to the manufacturer's instructions. The extracted DNA quantity and purity were estimated using Nanodrop (ThermoScientific) and used for PCR amplification with primers targeting the major capsid protein gene and DNA polymerase gene of LCDV (Table 1) [46-47]. PCR was performed using GoTaq Green Master Mix (Promega Madison, USA) in a total reaction volume of 25 µL containing 25 pmol of each primer, 10 µL DNA and 5 µL buffer. Cycling parameters included one denaturation step at 95°C for 3 min, followed by 30 amplification cycles at 95°C for 1 min, annealing at 54°C for 1 min, and extension of 72°C for 1 min. DNA from non-infected BF-2 cells was included as negative control. The amplified products were analyzed in 1.5% agarose-TAE gels containing ethidium bromide and visualized under UV light.

Table 1. *PCR Primers Used in the Study [46-47].*

Primer	Sequence
LF1[a]	F 5′ TTTGAATGGGAGGATCAC ′3
LR1[a]	R 5′ TCCGTAAATGCTGTTAGC ′3
LC1-F[b]	F 5′ AGGTTCAAGCGTCACAAG ′3
LC1-R[b]	R 5′ GGAAAACCCATTGATCCGT ′3

Two sets of PCR primers corresponding to DNA polymerase gene ([a]) and Major capsid protein gene ([b]) were used. The letters F and R depicts forward and reverse primers respectively.

3. Results and Discussion

The pectoral fins of the infected largemouth bass had a large number of irregular pinkish red clustered nodules (Figure 1), which is typical of an LCDV infection [6]. This virus is regarded as an integumental virus, affecting the skin, fins, and gills of host [1]. A strong tentative diagnosis of lymphocystis can be made based on the characteristic clinical sign of macroscopic nodules on host [28], which was evident in this case. Further microscopic examination of the wet mount preparation of fin nodules revealed clusters of lymphocystic (enormously hypertrophied) cells. These enlarged fibroblasts were easily visualized with a light microscope (Figure 2).

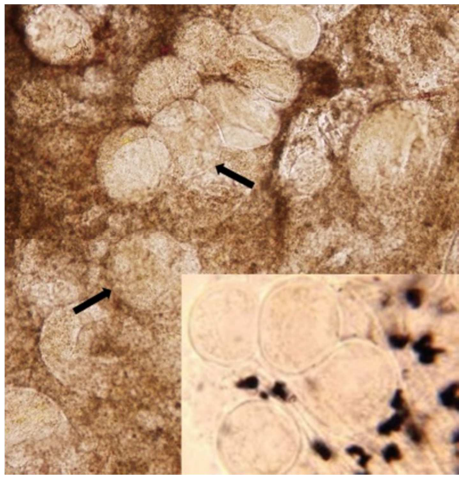

Figure 2. *Microscopic View of the Wet-Mount Preparation of Nodules from the Fin of the Infected Largemouth Bass. The nodules from the fin of the infected fish presented clusters of lymphocystic cells (arrow) by light microscopy. For comparison, the inset shows LCDV infected cells on the fins of a clownfish. Inset photo credit: Roy Yanong, UF/IFAS.*

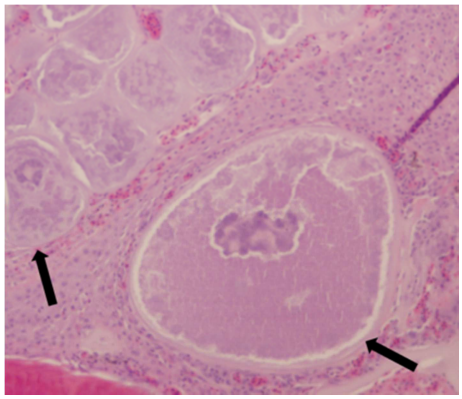

Figure 3. *Histological Sectioning (H&E staining) of a Nodule from the Fin of the Lymphocystis Virus Infected Largemouth Bass Depicting Cellular Hypertrophy. The infected cells exhibited cytomegaly (arrows) with irregularly-shaped intracytoplasmic inclusions. The basophilic inclusion bodies are surrounded by a thick hyaline capsule. Several adjacent hypertrophied cells are also visible.*

Histopathological analysis of the H&E stained nodular sections from the fin of the infected largemouth bass presented cellular hypertrophy characteristic of this viral infection (Figure 3). The infected cells showed irregularly shaped (ribbon-like), basophilic intracytoplasmic inclusions surrounded by a thick hyaline capsule. Similar features were observed previously in LCDV infected white spotted puffer [14]. The intracytoplasmic inclusions and hypertrophied cells are pathognomonic of LCDV infection [13-14] and were consistent with the present case. Several enlarged fibroblastic cells (~20,000 X) were visible compared to normal cells. Histopathological analysis of the lesions from the fins of the infected fish revealed several characteristics associated with LCDV such as severely hypertrophied cells, thick hyaline capsules surrounding the infected cells, and prominent basophilic intracytoplasmic inclusions [48].

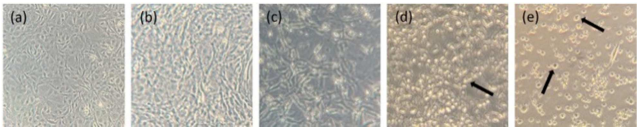

Figure 4. *Isolation of the LCDV Using BF-2 (Bluegill Fry) Cell line. The nodules from the fin of the infected fish were processed, filtered, and inoculated onto confluent monolayers of BF-2 cell line and incubated at 25°C. These were observed using a phase-contrast microscope. (a) Uninfected BF-2 cell line, (b) BF-2 cells 72 hrs, (c) 11 days, (d) 15 days, and e) 20 days post-inoculation. Cytopathic effects such as rounding of cells and destruction of cell monolayer (indicated by arrows) were observed in cells 15- and 20-days post-infection (d and e respectively).*

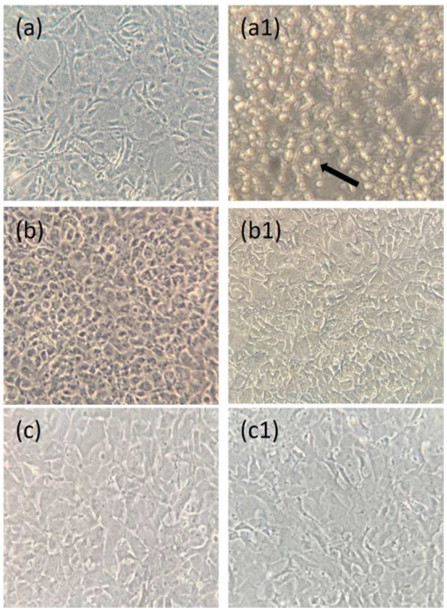

Figure 5. *Virus Isolation Using Fish Cell Lines. (a) BF-2, (b) EPC, and (c) FHM are uninfected controls. (a1) BF-2, (b1) EPC, and (c1) FHM cell monolayers were inoculated with processed samples from the fin nodules of the fish. The treated flasks were incubated at 25°C and observed for CPEs. The infected BF-2 cell line showed CPEs such as rounding of cells (arrow) and detachment of cells from the culture surface. No CPEs were observed in the EPC and FHM cell lines 15 days post-inoculation.*

Inoculation of the tissue filtrate from the fin nodules of the infected fish produced visible CPEs in BF-2 cells 15-days post-incubation confirming the presence of LCDV. The CPEs included rounding of cells, destruction of cell monolayer, and detachment of cells from the culture surface (Figures 4 and 5). The BF-2 cell line has been previously reported as susceptible to LCDV and used in the virus isolation [48]. Lymphocystis

virus from bluegill sunfish (*Lepomis macrochirus*) has been assayed using BF-2 cell line [48-49]. Both bluegill and largemouth bass belong to the family Centrarchidae allowing the virus proliferating in the BF-2 cell line as observed in the study (Figure 4). The virus is reported to replicate in fibroblastic cell lines and is best propagated in cells derived from the host or closely related species. The LCDV strains from rockfish (*Sebastes* sp.) and Japanese flounder (*P. olivaceus*) only infected host species from which they were isolated [23]. In addition, a strain of LCDV from a centrarchid required 40 days to generate lesions in a percid compared to 10 days in a centrarchid [1]. It is supported by the fact that no CPEs were observed in the EPC and FHM cell lines 15 days post-inoculation (Figure 5).

Ever since Nigrelli [50] first reported lymphocystis in striped bass at the New York Aquarium, it has been reported in wild and cultured striped bass. The aquarium from which the infected largemouth bass was collected had other species of fish that were uninfected. Several studies confirm that natural infection of LCDV can occur in one or two fish species from a family, even when other members are present [2, 27, 51]. This has been previously reported in centrarchids, particularly in *Micropterus* and *Lepomis*, which supports our observation.

DNA extracted from the cell cultures with evident CPEs or from affected tissues can be used for PCR-based assays [44-45]. The PCR amplification of the extracted DNA using primers targeting DNA polymerase and MCP gene did not yield anticipated products (data not shown) indicating either the primers used were not specific or the run settings need to be revised. However, the clustered nodules on the fins of the diseased fish, hypertrophied cells, inclusion bodies in the cell cytoplasm were pathognomonic of lymphocystis infection. Moreover, results from the cell line experiments supported that LCDV is the causative agent in the infected fish.

4. Conclusion

Lymphocystis disease virus infection was confirmed in the largemouth bass by means of histopathological analysis and the cell line studies. Histology of the infected fin nodules revealed encapsulated hypertrophic cells typical of the viral infection. The virus inoculated Bluegill Fry (BF-2) cells produced cytopathic effects 15-20 days post-inoculation.

Acknowledgements

The author thanks Dr. Lester Khoo (College of Veterinary Medicine, MSU) for the histopathology analysis of the infected fish tissues.

References

[1] Wolf, K. (1988). Chapter 22. Lymphocystis disease. In Fish Viruses and Fish Viral Diseases. Pp. 268-291. Cornell University Press. Ithaca, NY.

[2] Anders, K. (1989). Lymphocystis disease of fishes. Viruses of Lower Vertebrates 141-160.

[3] Marcogliese, D. J, Fournier M, Lacroix A, & Cyr D. G. (2001). Non-specific immune response associated with infections of lymphocystis disease virus in American plaice, *Hippoglossoides platessoides* (Fabricius). Journal of Fish Diseases, 24, 121-124.

[4] Iwamoto, R., Hasegawa O., LaPatra S. & Yoshimizu M. (2002). Isolation and characterization of the Japanese flounder (*Paralichthys olivaceus*) lymphocystis disease virus. Journal of Aquatic Animal Health, 14, 114-123.

[5] Cheng, S., Zhan W., Xing J., & Sheng X. (2006). Development and characterization of monoclonal antibody to the lymphocystis disease virus of Japanese flounder *Paralichthys olivaceus* isolated from China. Journal of Virology Methods, 135, 173-180.

[6] Noga, E. J. (2010). Problem 40. Lymphocystis. In Fish disease: diagnosis and treatment. 2nd edition. Wiley-Blackwell. Ames, Iowa. Pp. 171-173.

[7] Xu, L., Feng J., & Huang Y. (2014). Identification of lymphocystis disease virus from paradise fish *Macropodus opercularis* (LCDVPF). Archives of Virology, 159, 2445-2449.

[8] Huang, X, Huang Y, Xu L, Wei S, Ouyang Z, & Feng J. (2015). Identification and characterization of a novel lymphocystis disease virus isolate from cultured grouper in China. Journal of Fish Diseases, 38, 379-387.

[9] Doszpoly, A., G. L. Kaján, R. Puentes, & A. Perretta. (2020). Complete Genome Sequence and Analysis of a Novel Lymphocystivirus Detected in Whitemouth Croaker (*Micropogonias Furnieri*): Lymphocystis Disease Virus 4. Archives of Virology 165 (5), 1215–18.

[10] Perretta, A., Doszpoly, A., Puentes, R., & Bessonart, M. (2020). Diagnosis of lymphocystis disease in a novel host, the whitemouth croaker *Micropogonias furnieri*, associated with a putatively novel Lymphocystivirus species (LCDV-WC). Diseases of Aquatic Organisms, 137, 185-193.

[11] Kelly, D. C. & Robertson, J. S. (1973). Icosahedral cytoplasmic deoxyriboviruses. J. Gen Virol. 20, 17 - 41.

[12] Fenner, F. (1976). Classification and nomenclature of viruses. Second report of the International Committee on Taxonomy of Viruses. S. Karger; Basel, London.

[13] Alonso, M. C., Cano I., Garcia-Rosado E., Castro D., Lamas J., Barja J. L. & Borrego J. J. (2005). Isolation of lymphocystis disease virus (LCDV) from sole, *Solea senegalensis* Kaup, and blackspot sea bream, *Pagellus bogaraveo* (Brünnich). Journal of Fish Diseases, 28, 221-228.

[14] Sheng, X. Z, Zhan W. B., & Wang Y. (2007). White spotter puffer *Arothron hispidus*, a new host for lymphocystis in Qingdao Aquarium of China. Diseases of Aquatic Organisms, 75, 23-28.

[15] Paperna, I, Vilenkin., & de Matos A. P. (2001). Iridovirus infections in farm-reared tropical ornamental fish. Diseases of Aquatic Organisms 48, 17-25.

[16] Menezes, J., Ramos M. A., & Oereira T. G. (1987). Lymphocystis disease: an outbreak in *Sparus aurata* from Ria Formosa, south coast of Portugal. Aquaculture, 67, 222-225.

[17] Basurco, A., Tiana A., Castellanos J. V., & Tarazona M. J. (1990). First report of lymphocystis disease in *Sparus aurata* (Linnaeus) in Spain. Bull. Eur. Assoc. Fish Pathology, 10, 71-73.

[18] Moate, R. M., Harris J. E., & McMahon S. (1992). Lymphocystis infections in cultured gilt-head sea bream (*Sparus aurata*) in the Aegean Sea. Bull. Eur. Assoc. Fish Pathology, 12, 134-136.

[19] Le Deuff, R. M., & Renault T. (1993). Lymphocystis outbreaks in farmed sea bream, *Sparus aurata*, first report on French Mediterranean. Bull. Eur. Assoc. Fish Pathology, 13, 130-133.

[20] Garcia-Rosado, E., Castro, D., Rodriguez, S., Perez-prieto, S. I., & Borrego, J. J. (1999). Isolation and characterization of lymphocystis virus (FLDV) from gilt-head sea bream (*Sparus aurata*, L.) using a new homologous cell line. Bull. Eur. Ass. Fish Pathology, 19, 53-56.

[21] Berthiaume, L, Alain R., & Robin J. (1984). Morphology and ultrastructure of lymphocystis disease virus, a fish iridovirus, grown in tissue culture. Virology, 135, 10-19.

[22] Tidona, C. A., & Darai G. (1999). Lymphocystis disease virus (Iridoviridae). In: Encyclopedia of Virology (ed. by A. Granoff & R. G. Webster), pp. 908– 911. Academic Press, New York.

[23] Kitamura, S. I., Jung S. J., Kim W. S., Nishizawa T., Yoshimizu M., & Oh M. J. (2006a). A new genotype of lymphocystivirus, LCD-RF, from lymphocystis disease rockfish. Archives of Virology, 151, 607-615.

[24] Tidona, C. A., & Darai G. (1997). The complete DNA sequence of lymphocystis disease virus. Virology, 230, 353-362.

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

[26] Jancovich, J. K., Chinchar, V. G., Hyatt, A., Miyazaki, T., Williams, T., & Zhang, Q. Y. (2012). Family Iridoviridae. In: King, A. M. Q., Adams, M. J., Carstens, E. B., Lefkowitz, E. J. (Eds.), Virus Taxonomy, Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, CA.

[27] Cano, I., Ferro P., Alonso M. C., Bergmann S. M., Romer-Oberdorfer A., Garcia R. E., Castro D., & Borrego J. J. (2006a). Development of molecular techniques for detection of lymphocystis disease virus in different marine fish species. Journal of Applied Microbiology, 102, 32-40.

[28] Nigrelli, R. F., & Smith G. M. (1939). Studies on lymphocystis disease in the orange filefish, *Ceratacanthus schoepfi* (Walbaum) from Sandy Hook Bay, New Jersey. Zoologica 24, 255-264.

[29] Roberts, R. J., & Ellis, A. E. (2012). The anatomy and physiology of teleosts. In: Roberts, R. J. (Ed.), Fish Pathology. fourth ed Wiley-Blackwell, Ames, pp. 17–61.

[30] Pritchard, H. N. & Malsberger, R. G. (1968). A cytochemical study of lymphocystis tumor cells in vivo. J. exp. Zoology. 169, 371-380.

[31] Samalecos, C. P. (1986). Analysis of the structure of fish lymphocystis disease virions from skin tumors of Pleuronectes. Archives of Virology, 91, 1-10.

[32] Ferguson, H. W. (2006). Skin. In: Ferguson, H. W. (Ed.), Systemic Pathology of Fish. second ed. Scotian Press, London, pp. 65–89.

[33] Paperna, I., Sabnai I., & Colorni A. (1982). An outbreak of lymphocystis in *Sparus aurata* L. in the Gulf of Aqaba, Red Sea. Journal of Fish Diseases, 5, 433-437.

[34] Kitamura, S. I., Ko J. Y., Lee W. L., Kim S. R., Song J. Y., Kim D. K., Jung S. J. & Oh M. J. (2007). Seasonal prevalence of lymphocystis disease virus and aquabirnavirus in Japanese flounder, *Paralichthys olivaceus*, and blue mussel, *Mytilus galloprovincialis*. Aquaculture, 266, 26-31.

[35] Cano, I, Valverde E. J., Lopez-Jimena B., Alonso M. C., & Garcia-Rosado E. (2010). A new genotype of Lymphocystivirus isolated from cultured gilthead seabream, *Sparus aurata* L., and Senegalese sole, *Solea senegalensis* (Kaup). Journal of Fish Diseases, 33, 695-700.

[36] Dezfuli, B. S., Lui A., Giari L., CastaldelliMulero V., & Noga E. J. (2012). Infiltration and activation of acidophilic granulocytes in skin lesions of gilthead seabream, *Sparus aurata*, naturally infected with lymphocystis disease virus. Dev Comp Immunol 36, 174-182.

[37] Haddad-Boubaker, S., Bouzgarou N., FakhfakhE, Khayech M., & Ben Mohamed S. (2013). Detection and Genetic Characterization of Lymphocystis Disease Virus (LCDV) Isolated during Disease Outbreaks in Cultured Gilt-head Sea Bream *Sparus aurata* in Tunisia. Fish Pathology, 48, 101-104.

[38] Oh, M. J., Kitamura S. I., Kim W. S., Park M. K., Jung S. J., Miyadai T., & 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.

[39] Colorni, A., & Padros, F. (2011). Diseases and health management. In: Pavlidis, M. A., Mylonas, C. C. (Eds.), Sparidae: Biology and Aquaculture of Gilthead Sea Bream and Other Species. Wiley-Blackwell, Oxford, pp. 321-357.

[40] Schnitzler, P., & Darai G. (1993). Identification of the gene encoding the major capsid protein from fish lymphocystis disease virus. Journal of General Virology, 74, 2143-2150.

[41] Tidona, C. A., Schnitzler P., Kehm R., & Darai G. (1998). Is the major capsid protein of iridoviruses a suitable target for the study of viral evolution? Virus Genes, 16, 59-66.

[42] Hanson, L. A., Rudis M. R., Vasques-Lee M., & Montgomery R. D. (2006). A broadly applicable method to characterize large DNA viruses and adenoviruses based on the DNA polymerase gene. Journal of Virology, 11 (3), 8.

[43] Hossain, M., Song J. Y, Kitamura S. I, Jung S. J, & 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.

[44] Weber ES, III, Waltzek TB, Young DA, Twitchell EL, Gates AE, Vagelli A, Risatti GR, Hedrick RP, & Frasca S., Jr. (2009). Systemic iridovirus infection in the Banggai cardinalfish (*Pterapogon kauderni* Koumans 1933). J Vet Diagn Invest, 21, 306–320.

[45] Whittington R. J, Becker JA, & Dennis MM. (2010). Iridovirus infections in finfish: Critical review with emphasis on ranaviruses. Journal of Fish Diseases, 33, 95–122.

[46] Kitamura, S. I., Jung S. J., & Oh M. J. (2006b). Differentiation of lymphocystis disease virus genotype by multiplex PCR. Journal of Microbiology, 44, 248-253.

[47] Kvitt, H., Heinisch, G., & Diamant, A. (2008). Detection and phylogeny of Lymphocystis virus in sea bream *Sparus aurata* based on the DNA polymerase gene and major capsid protein sequences. Aquaculture, 275, 58-63.

[48] Wolf, K., Gravell M., & Malsberger R. G. (1966). Lymphocystis virus: isolation and propagation in centrarchid fish cell lines. Science, 151, 1004-1005.

[49] Wolf, K., & Carlson C. P. (1965). Multiplication of lymphocystis virus in the bluegill (*Lepomis macrochirus*). Ann NY Acad Sci 126, 414-419.

[50] Nigrelli, R. F. (1940). Mortality statistics for specimens in the New York aquarium, 1939. Zoologica, 25, 525-552.

[51] Palmer, L. J., Hogan N. S., & Van den Heuvel M. R. (2012). Phylogenetic analysis and molecular methods for the detection of lymphocystis disease virus from yellow perch, *Perca flavescens* (Mitchell). Journal of Fish Diseases, 35, 661-670.