Persistence of Lymphocystis Disease Virus (LCDV) in Seawater

Rocío Leiva-Rebollo[1] · Alejandro M. Labella[1] · Estefanía J. Valverde[1] · Dolores Castro[1] · Juan J. Borrego[1]

Abstract
Lymphocystis disease virus (LCDV), the causative agent of lymphocystis disease (LCD), is a waterborne pathogen that uses the external surfaces, including the gills, as portals to gain access to fish host. However, there are no data on LCDV persistence in the aquatic environment. In this study, the persistence of LCDV in natural (raw), treated (autoclaved and filtered) and synthetic seawater held at 22 and 18 °C has been evaluated. The estimated T_{99} values for LCDV in seawater ranged from 2.7 to 242 days depending on seawater type and temperature, with the highest value recorded at 22 °C in autoclaved seawater. Microbiota and temperature seem to be the main factors affecting the persistence of LCDV in seawater. The results indicated that LCDV is more stable in treated seawater than most of the fish pathogenic viruses studied so far, supporting the relevance of this medium for the prevalence of LCD in fish farms.

Keywords Lymphocystis disease virus · Persistence · Seawater · Temperature · Microbiota · Abiotic factors

Introduction

Waterborne transmission is the most relevant route for regional or local spread of fish viral pathogens (Oidtmann et al. 2014; Foreman et al. 2015). For successful waterborne transmission, virus must retain infectivity for sufficient time to reach and infect a susceptible host (Garver et al. 2013; Munro et al. 2016). Therefore, data on viral persistence in the aquatic environment allow to determine the risk level that virus represents for the aquaculture practice (Oidtmann et al. 2017). Common trends applied to the persistence of fish pathogenic viruses in water: (i) virus viability decreases with increasing temperature (Kim et al. 2012; Moser et al 2012; Toffan et al. 2016); (ii) suspended soils negatively affect the infectivity of viruses (Yoshimizu et al. 1986; Yoshinaka et al. 2000); (iii) virus decay is a function of time (Oidtmann et al. 2017); and (iv) the inactivation of viruses in natural water seems to be related to microbiota present in these waters (Kamei et al. 1988b; Kimura et al. 1990; Yoshimizu et al. 2005).

Lymphocystis disease (LCD) is a well-known disease caused by the iridovirus lymphocystis disease virus (LCDV) that affects more than 125 fish species from both marine and freshwater environments (Anders 1989; Plumb 1993). The typical sign of LCD is the presence of small nodular lesions, usually grouped in raspberry-like clusters of tumorous appearance, on the fish skin and fins, that may cover the entire body in severely affected individuals (Smail and Munro 2001; Borrego et al. 2017).

The prevalence of LCD in aquaculture facilities depends on several factors, including fish density, human manipulation, temperature, salinity and oxygen content of the water, and immunological status of fish (Berthiaume et al. 1993; Sindermann 1996; Kitamura et al. 2007; Colorni and Padrós 2011; Hick et al. 2016). LCD outbreaks in marine farms occur mainly at temperatures ranging between 20 and 27 °C, depending on fish species (Paperna et al. 1982; Xing et al. 2006; Kvitt et al. 2008; Hossain et al. 2009). Diseased fish show low growth rates, which may be caused by the anaemia generally associated with LCD (Iwamoto et al. 2002); however, mortalities are typically limited to those fish whose swimming, breathing or feeding are severely impaired by a gross development of lymphocystis lesions (Colorni and Padrós 2011). Moreover, LCD may favour secondary bacterial infections and/or parasitic infestations, increasing fish mortality rates (Borrego et al. 2017).

Direct contact and waterborne transmission are the routes accepted for the spreading of LCDV infections; the external surfaces including the gills appear to be the main portals

of viral entry (Wolf 1988; Bowser et al 1999; Kvitt et al. 2008). In addition, LCDV has been experimentally transmitted through the oral route (Wang et al. 2007; Cano et al. 2013; Carballo et al. 2019), and there are strong evidences of vertical transmission, with LCDV carried on the egg surface (Sun et al. 2003; Cano et al. 2013). Viral contamination of food and eggs probably derive from LCDV present in the rearing seawater (Cano et al. 2009, 2013).

Little is known on the fate of LCDV outside the host and whether the virus is able to remain infective for an extended period of time in water. Therefore, the objective of this work was to study the influence of temperature, and the biotic and abiotic factors present in seawater on the persistence of LCDV. This information could be used in conjunction with infectious dose data to make epidemiological inferences and to inform management and control strategies for LCD outbreaks.

Materials and Methods

Virus Propagation and Quantification

LCDV strain Leetown NFH (ATCC VR-342), used as model virus, was propagated in the BF-2 cell line. Cells were cultured at 25 °C in Leibovitz's L-15 medium (Gibco, Life Technologies, Carlsbad, CA, USA) supplemented with 2% L-glutamine (Sigma-Aldrich, St. Louis, MO, USA), 1% penicillin–streptomycin (Sigma-Aldrich) and 10% foetal bovine serum (FBS) (Gibco). Semi-confluent cell monolayers in 75 cm^2 Nunc Easyflasks (Thermo Fisher Scientific, Waltham, MA, USA) were inoculated with 200 µl of a 1:200 dilution (in L-15 medium) of the viral stock, incubated at 20 °C and maintained until cytopathic effects (CPE) were observed in more than 75% of the cells. At this time, cells were harvested, washed twice with PBS (0.1 M, pH 7.4) and lysed by three freeze–thaw cycles (liquid nitrogen-room temperature). Cells debris were removed by centrifugation at 5000×*g* for 10 min at 4 °C, and the supernatant containing the virus was stored at − 80 °C until use.

Viral titration was performed by endpoint dilution assay using BF-2 cells. Semi-confluent cell monolayers in 24-well plates (Nunc, Thermo Fisher Scientific) were inoculated in triplicate with 200 µl per well of the appropriate viral dilutions. After 2-h adsorption at 20 °C, the inoculum was removed and replaced with 1 ml of maintenance medium (L-15 medium with 2% FBS). Inoculated cells were incubated at 20 °C and maintained up to 14 day to observe CPE. The 50% cell culture infectious dose ($TCID_{50}$) values were calculated using the Reed and Muench method (Reed and Muench 1938). The minimum detectable limit of the assay was 10 $TCID_{50}$ per ml.

Survival Studies

The persistence of LCDV was determined in raw seawater (RSW), seawater sterilized by autoclaving at 121 °C for 15 min (ASW), and seawater filtered through 0.45-µm pore-size mixed cellulose esters membrane filter (Millipore Iberica, Madrid, Spain) (FSW). In addition, autoclaved synthetic seawater (SSW) was used as control medium. SSW was prepared using 33 g/l of Instant Ocean (Aquarium Systems, Sarrebourg, France) in Milli-Q water. Salinity, estimated by conductivity measures using a portable conductivity meter (Crison, Barcelona, Spain), was 29.7 ppt for RSW, FSW and ASW, and 24.4 ppt for SSW. The pH of the seawater samples at time zero ranged from 8.0 to 8.7 for all seawater types tested.

One hundred ml Erlenmeyer flasks containing 50 ml of each seawater type were inoculated with the LCDV stock at a viral titre of 10^7 $TCID_{50}$/ml for RSW and 10^5 $TCID_{50}$/ml for the other seawater types, and then, they were incubated at two different temperatures (22 and 18 °C) at constant 100 rpm orbital shaking in the dark. All the experiments were performed in triplicate, and sampling was carried out within the first 30-min post-inoculation (p.i.) (time 0) and after 1, 3, 7, 15, 30, 90, and 180 days p.i. for each treatment (4 seawater types and 2 temperatures). Aliquots of 400 µl were sampled for the titration of infectious virus by the endpoint dilution assay.

Seawater types were tested for cytotoxicity on BF-2 cells. Semi-confluent cell monolayers in 24-well plates were inoculated with 200 µl aliquots of each seawater type and incubated at 20 °C for 15 days. Microbiota growth and toxic effects were observed on BF-2 cells inoculated with RSW. For this reason, an antimicrobial treatment was applied to samples of LCDV-inoculated RSW prior to viral titration. Penicillin–streptomycin and amphotericin B (Sigma-Aldrich) solutions were used at different concentrations (from 1 to 5%) and contact times (6, 12 and 24 h). The antimicrobial treatment was evaluated in terms of cytotoxicity on BF-2 cells; microbiota inhibition, determined by reduction of the bacterial colony count on Difco Marine agar 2216 (BD Life Sciences, Mississauga, ON, Canada) plates incubated at 20 °C, and effect on the LCDV infectious titre in BF-2 cells. On the basis of the results obtained, a final concentration of 200 IU/ml penicillin, 200 µg/ml streptomycin and 5 µg/ml amphotericin B during 6 h was used as pre-treatment of the samples of RSW to determine the survival of LCDV.

Statistical Analysis

The results were expressed as mean viral titre ± standard deviation. Kruskal–Wallis test (α = 0.05) was applied to compare the viral titres obtained during the course of the study for each treatment, as well to compare different types of treatments at each time point. Multiple pairwise comparisons were carried out by using Dunn's procedure. Statistical analyses were performed using XLSTAT version 2018.2 software. First-order inactivation rate constant (k) was computed by performing linear regression analysis on plots of ln (N_t/N_0) versus t, where N_0 equals the initial infectious titre and N_t equals the titre at elapsed time t (in days). T_{99} values, time in days for a 2-log reduction of the viral titre, were calculated by extrapolating in the inactivation functions.

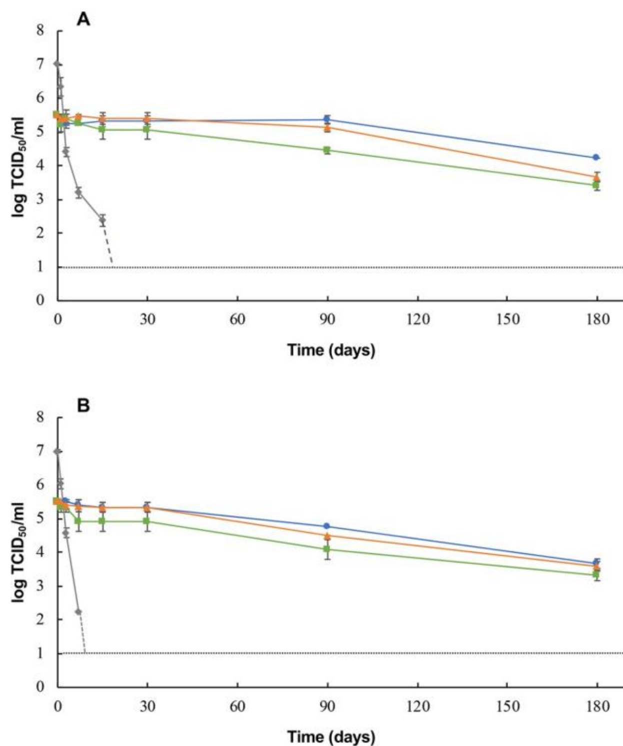

Fig. 1 Inactivation kinetics of LCDV strain VR-342 at 22 °C (**a**) and 18 °C (**b**) in raw seawater (RSW) (diamonds), autoclaved seawater (ASW) (circles), filtered seawater (FSW) (squares) and autoclaved synthetic seawater (SSW) (triangles). Errors bars represent standard deviation based on triplicate assays. Dotted line represents limit of detection

Results

The temporal evolution of LCDV strain VR-342 infectious titre in the different experimental conditions studied (seawater type and temperature) is shown in Fig. 1. Viral infectivity was maintained (no significant differences, $p < 0.05$) in treated (ASW and FSW) and autoclaved synthetic seawater (SSW) during the first 30 day (90 day in the case of virus suspended in ASW at 22 °C) at both 22 and 18 °C, showing afterwards a decrease that can be assumed to be a first-order process (R^2 values higher than 0.93), with reductions of infectious titres smaller than 2 logs after 180 day. No significant differences were observed in viral titres at the end of the experiment for any of the treatments, except for the virus suspended in ASW at 22 °C ($p < 0.05$). The inactivation kinetic of LCDV in RSW was faster than in the other seawater types, and the infectious titres dropped below the detection limit after 7 and 15 days at 18 and 22 °C, respectively.

The estimated inactivation rate constant (k) and T_{99} values are presented in Table 1. The k value for LCDV strain VR-342 was 0.03 day^{-1} for all treated seawater types, whereas T_{99} values ranged from 157 day in FSW at 18 °C and 242 day in ASW at 22 °C, being higher at 22 °C than at 18 °C for each treated seawater type. In the case of RSW, the inactivation of the virus at 18 °C was 2.25 times faster than at 22 °C, with T_{99} values of 2.7 and 4 day at 18 and 22 °C, respectively.

Discussion

Although a number of studies concerning the survival of fish pathogenic viruses in seawater have been published (Toranzo and Hetrick 1982; Barja et al. 1983; Kamei et al. 1988a; Yoshimizu et al. 2005; Hawley and Garver 2008; Oidtmann et al. 2017), no information is available at present on the persistence of LCDV in the aquatic environment.

Table 1 Least square fit computed inactivation rate constants (k) and T_{99} values for LCDV strain VR-342 in different seawater types incubated at 22 and 18 °C

	22 °C				18 °C			
	RSW	ASW	FSW	SSW	RSW	ASW	FSW	SSW
k (day^{-1})	0.678	0.027	0.027	0.028	1.528	0.027	0.027	0.028
T_{99} (day)	4	242	172	203	2.7	195	157	183
R^2	0.815	1	0.999	0.936	0.995	0.999	0.964	0.989

RSW raw seawater, *ASW* autoclaved seawater, *FSW* filtered seawater, *SSW* autoclaved synthetic seawater, R^2 coefficient of determination

The results obtained in our study demonstrate that LCDV can persist for an extended period under certain laboratory conditions. Furthermore, LCDV persistence is significantly influenced by the type of seawater and water temperature. The temperatures chosen in the present study represent the mean seawater temperatures in fall-spring (18 °C) and summer (22 °C) in our geographical area, the latter corresponds with the minimal temperature associated with LCD outbreaks in gilthead seabream (*Sparus aurata*) farms in the Mediterranean basin (Colorni and Padrós 2011). Viral infectivity decays very slowly in treated and synthetic seawater, with infectious titres over 10^3 $TCID_{50}$/ml at the end of the experiment. The T_{99} values calculated for LCDV in these seawater types are markedly higher (months versus days or weeks) than those reported for the majority of relevant fish viruses, both in freshwater and seawater, and only comparable with the persistence of infectious pancreatic necrosis virus (IPNV) in freshwater (Oidtmann et al. 2017). However, LCDV persistence in raw seawater was shorter than that obtained in treated seawater, being similar to that reported for other fish viruses (Afonso et al. 2012; Kell et al. 2014). Interestingly, LCDV viability is higher at 22 °C than at 18 °C, which is the opposite situation to that described for most viruses (Toranzo and Hetrick 1982; Barja et al. 1983; Hawley and Garver 2008; Tapia et al. 2013), including the ranavirus frog virus 3, the only iridovirus that has been studied so far (Nazir et al. 2012; Munro et al 2016). A possible explanation of the different effect of temperature on LCDV persistence is that LCDV might present a better infectivity or replication on host cells at high temperature (20 °C or higher) (Kitamura et al. 2007; Hossain et al. 2009; Moser et al. 2012). Thus, Paperna et al. (1982) and Xing et al. (2006) noted that the occurrence of LCD in fish farms depended on the increase of water temperature at > 20 °C. Water temperature becomes one of the key factors for viral replication capacity, as it has been described for several viral fish pathogens (Vo et al. 2015; Toffan et al. 2016).

There is clear evidence that the type of water and treatment of the water prior to virus seeding have a substantial impact on viral persistence (Kamei et al. 1988a; Shimizu et al. 2006; Hawley and Garver 2008; Garver et al. 2013). Inactivation times in filtered and autoclaved seawater were longer compared to raw seawater, which can be attributed to the presence of microbiota, mainly bacteria, with antiviral activity (Kamei et al. 1988b; Kimura et al. 1990; Yoshimizu and Ezura 1999; Yoshimizu et al. 2005; Shimizu et al. 2006).

We have used three seawater types: filtered seawater, autoclaved seawater, and synthetic seawater to obtain certain information on the influence of abiotic factors on LCDV persistence. The persistence of LCDV was significantly higher in ASW compared to FSW at both temperatures, and to SSW at 22 °C. These results could be explained by the presence of a filterable antagonistic to virus survival in raw seawater. Both proteolytic enzymes and heavy metals have been proposed as filterable virucidal agents that can be eliminated by autoclaving (Gerba and Schaiberger 1975; Kapuscinski and Mitchell 1980). Alternatively, particulate matter or sediments present in seawater, but not in synthetic seawater, may have a protective effect on virus infectivity that can be removed by filtration (Yoshinaka et al. 2000; Yoshimizu et al. 2005). This protective effect may result from the adsorption of viral inactivating agents or the virus itself to particulate matter (Gerba and Schaiberger 1975).

Evaluating the risk of waterborne transmission requires knowledge of the rate of viral shedding from infected animals, the stability of virus in seawater and the minimal infectious dose to produce the disease (Foreman et al. 2015). None of these key parameters have been estimated for LCDV infections. Nevertheless, the persistence observed in this study, combined with the infectious titres retained after a 6-month period in treated seawater, supports the relevance of this transmission route for the prevalence of LCD in fish farms.

In short, LCDV persists for prolonged periods in treated seawater, which it is generally used in the aquaculture practice, with higher survival times at 22 °C. The stability of LCDV in treated seawater highlights the importance of establishing biosecurity measures to prevent the spread of LCDV through the aquatic environment in the fish farms.

Acknowledgements This study was supported by a project from Junta de Andalucía (P12-RNM-2261) granted to J.J. Borrego. R. Leiva-Rebollo was supported by a fellowship from Junta de Andalucía. The authors thank Cristina Zaballa for her collaboration in carrying out the evaluation of the antimicrobial treatment.

References

Afonso, L. O. B., Richmond, Z., Eaves, A. A., Richard, J., Hawley, L. M., & Garver, K. A. (2012). Use of ultraviolet C (UVC) radiation to inactivate infectious hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV) in fish processing plant effluent. *Journal of Aquaculture Research and Development, 3*, 1–5.

Anders, K. (1989). Lymphocystis disease of fishes. In W. Ahne & D. Kurstak (Eds.), *Viruses of lower vertebrates* (pp. 141–160). Berlin: Springer.

Barja, J., Toranzo, A., Lemos, M., & Hetrick, F. (1983). Influence of water temperature and salinity on the survival of IPN and IHN viruses. *Bulletin of the European Association of Fish Pathologists, 3*, 47–50.

Berthiaume, L., Heppel, J., Desy, M., Leblanc, L., Lallier, R., Bailey, R., et al. (1993). Manifestation of lymphocystis disease in American plaice (*Hippoglossoides platessoides*) exposed to low salinities. *Canadian Journal of Fisheries and Aquatic Sciences, 50*, 430–434.

Borrego, J. J., Valverde, E. J., Labella, A. M., & Castro, D. (2017). Lymphocystis disease virus: Its importance in aquaculture. *Reviews in Aquaculture, 9*, 179–193.

Bowser, P. R., Wooster, G. A., & Getchell, R. G. (1999). Transmission of walleye dermal sarcoma and lymphocystis via waterborne exposure. *Journal of Aquatic Animal Health, 11*, 158–161.

Cano, I., Lopez-Jimena, B., Garcia-Rosado, E., Ortiz-Delgado, J. B., Alonso, M. C., Borrego, J. J., et al. (2009). Detection and persistence of Lymphocystis disease virus (LCDV) in *Artemia* sp. *Aquaculture, 291*, 230–236.

Cano, I., Valverde, E. J., Garcia-Rosado, E., Alonso, M. C., Lopez-Jimena, B., Ortiz-Delgado, J. B., et al. (2013). Transmission of lymphocystis disease virus to cultured gilthead seabream, *Sparus aurata* L., larvae. *Journal of Fish Diseases, 36*, 569–576.

Carballo, C., Ortiz-Delgado, J. B., Berbel, C., Castro, D., Borrego, J. J., Sarasquete, C., et al. (2019). Feed and immersion challenges with lymphocystis disease virus (LCDV) reveals specific mechanisms for horizontal transmission and immune response in Senegalese sole post-larvae. *Fish and Shellfish Immunology, 89*, 710–718.

Colorni, A., & Padrós, F. (2011). Diseases and health management. In M. A. Pavlidis & C. C. Mylonas (Eds.), *Sparidae: Biology and aquaculture of gilthead sea bream and other species* (pp. 321–357). Oxford: Wiley-Blackwell.

Foreman, M. G. G., Guo, M., Garver, K. A., Stucchi, D., Chandler, P., Wan, D., et al. (2015). Modelling infectious hematopoietic necrosis virus dispersion from marine salmon farms in the Discovery Islands, British Columbia, Canada. *PLoS ONE, 10*, e0130951.

Garver, K. A., Mahony, A. A. M., Stucchi, D., Richard, J., Van Woensel, C., & Foreman, M. (2013). Estimation of parameters influencing waterborne transmission of infectious hematopoietic necrosis virus (IHNV) in Atlantic Salmon (*Salmo salar*). *PLoS ONE, 8*, e82296.

Gerba, C. P., & Schaiberger, G. E. (1975). Effect of particulates on virus survival in seawater. *Journal Water Pollution Control Federation, 47*, 93–103.

Hawley, L. M., & Garver, K. A. (2008). Stability of viral haemorrhagic septicemia virus (VHSV) in freshwater and seawater at various temperatures. *Diseases of Aquatic Organisms, 82*, 171–178.

Hick, P., Becker, J., & Whittington, R. (2016). Iridoviruses of fish. In F. S. B. Kibenge & M. Godoy (Eds.), *Aquaculture virology* (1st ed., pp. 127–152). Cambridge, MA: Academic Press.

Hossain, M., Kim, S. R., Kitamura, S. I., Kim, D. W., Jung, S. J., Nishizawa, T., et al. (2009). Lymphocystis disease virus persists in the epidermal tissues of olive flounder, *Paralichthys olivaceus* (Temminch & Schlegel), at low temperatures. *Journal of Fish Diseases, 32*, 699–703.

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.

Kamei, Y., Yoshimizu, M., Ezura, Y., & Kimura, T. (1988a). Effects of environmental water on the infectivities of infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV). *Journal of Applied Ichthyology, 4*, 37–47.

Kamei, Y., Yoshimizu, M., Ezura, Y., & Kimura, T. (1988b). Screening of bacteria with antiviral activity from fresh water salmonid hatcheries. *Microbiology and Immunology, 32*, 67–73.

Kapuscinski, R. B., & Mitchell, R. (1980). Process controlling virus inactivation in coastal waters. *Water Research, 14*, 363–371.

Kell, A. M., Wargo, A. R., & Kurath, G. (2014). Viral fitness does not correlate with three genotype displacement events involving infectious hematopoietic necrosis virus. *Virology, 464*, 146–155.

Kim, S. J., Si, J., Lee, J. E., & Ko, G. (2012). Temperature and humidity influences on inactivation kinetics of enteric viruses on surfaces. *Environmental Science and Technology, 46*, 13303–13310.

Kimura, T., Yoshimizu, Y., Ezura, Y., & Kamei, Y. (1990). An antiviral agent (46NW-04A) produced by *Pseudomonas* sp. and its activity against fish viruses. *Journal of Aquatic Animal Health, 2*, 12–20.

Kitamura, S. I., Ko, J. Y., Lee, W. L., Kim, S. R., Song, J. Y., Kim, D. K., et al. (2007). Seasonal prevalence of lymphocystis disease virus and aquabirnavirus in Japanese flounder, *Paralichthys olivaceus* and blue mussel, *Mytilus galloprovincialis*. *Aquaculture, 266*, 26–31.

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

Moser, J. R., Galván Álvarez, D. A., Mendoza Cano, F., Encinas Garcia, T., Coronado Molina, D. E., Portillo Clark, G., et al. (2012). Water temperature influences viral load and detection of White Spot Syndrome Virus (WSSV) in *Litopenaeus vannamei* and wild crustaceans. *Aquaculture, 326–329*, 9–14.

Munro, J., Bayley, A. E., McPherson, N. J., & Feist, S. W. (2016). Survival of frog virus 3 in freshwater and sediment from an English lake. *Journal of Wildlife Diseases, 52*, 138–142.

Nazir, J., Spengler, M., & Marschang, R. E. (2012). Environmental persistence of amphibian and reptilian ranaviruses. *Diseases of Aquatic Organisms, 98*, 177–184.

Oidtmann, B., Dixon, P., Way, K., Joiner, C., & Bayley, A. E. (2017). Risk of waterborne virus spread: Review of survival of relevant fish and crustacean viruses in the aquatic environment and implications for control measures. *Reviews in Aquaculture*. https://doi.org/10.1111/raq.12192.

Oidtmann, B. C., Peeler, E. J., Thrush, M. A., Cameron, A. R., Rees, R. A., Pearce, F. M., et al. (2014). Expert consultation on risk factors for introduction of infectious pathogens into fish farms. *Preventive Veterinary Medicine, 115*, 238–254.

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.

Plumb, J. A. (1993). Viral diseases of marine fish. In J. A. Couch & J. W. Fournie (Eds.), *Pathobiology of marine and estuarine organisms* (pp. 25–52). Boca Raton, FL: CRC Press.

Reed, L. J., & Muench, H. (1938). A simple method of estimating fifty per cent endpoints. *American Journal of Hygiene, 27*, 493–497.

Shimizu, T., Yoshida, N., Kasai, H., & Yoshimizu, M. (2006). Survival of koi herpesvirus (KHV) in environmental water. *Fish Pathology, 41*, 153–157.

Sindermann, C. J. (1996). Pollution and infectious diseases. In C. J. Sindermann (Ed.), *Ocean pollution: Effects on living resource and humans* (pp. 37–40). Boca Raton, FL: CRC Press.

Smail, D. A., & Munro, A. L. S. (2001). The virology of teleosts. In R. J. Roberts (Ed.), *Fish pathology* (3rd ed., pp. 169–253). Orlando, FL: W.B. Saunders.

Sun, X. Q., Huang, J., Liu, Y. K., Qu, L. Y., Hong, X. G., & Zhang, J. X. (2003). The studies on diagnostic method of dot blot and in situ hybridization for lymphocystis disease of cultured *Paralichthys olivaceus*. *High Technology Letters, 1*, 89–94.

Tapia, E., Monti, G., Rozas, M., Sadoval, A., Gaete, A., Bohle, H., et al. (2013). Assessment of the in vitro survival of the infectious salmon anaemia virus (ISAV) under different water types and temperatures. *Bulletin of the European Association of Fish Pathologists, 33*, 3–12.

Toffan, A., Panzarin, V., Toson, M., Cecchettin, K., & Pascoli, F. (2016). Water temperature affects pathogenicity of different betanodavirus genotypes in experimentally challenged *Dicentrarchus labrax*. *Diseases of Aquatic Organisms, 119*, 231–238.

Toranzo, A. E., & Hetrick, F. M. (1982). Comparative stability of two salmonid viruses and poliovirus in fresh, estuarine and marine waters. *Journal of Fish Diseases, 5*, 223–231.

Vo, N. T. K., Bender, A. W., Lee, L. E. J., Lumsden, J. S., Lorenzen, N., Dixon, B., et al. (2015). Development of a walleye cell line and use to study the effects of temperature on infection by viral

haemorrhagic septicaemia virus group IVb. *Journal of Fish Diseases, 38*, 121–136.
Wang, M., Lin, X., Ma, G., & Bai, X. (2007). Emerging viral diseases of fish and shrimp. In S. K. Lal (Ed.), *Emerging viral diseases of Southeast Asia Issues in infectious diseases* (vol. 4, pp. 35–58). Basel: Karger.
Wolf, K. (1988). *Fish viruses and fish viral diseases*. Ithaca, NY: Cornell University Press.
Xing, J., Sheng, X., & Zhan, W. (2006). *Lymphocystis disease and diagnostic methods in China* (pp. 30–33). XI: Aquaculture Asia Magazine.
Yoshimizu, M., & Ezura, Y. (1999). Biological control of fish viral diseases ny anti-viral substance producing bacteria. *Microbes and Environment, 14*, 269–275.
Yoshimizu, M., Takizawa, H., Kamei, Y., & Kimura, T. (1986). Interaction between fish pathogenic viruses and microorganisms in fish rearing water: Survival and inactivation of infectious hematopoietic necrosis virus, infectious pancreatic necrosis virus and *Oncorhynchus masou* virus in rearing water. *Fish Pathology, 21*, 223–231.
Yoshimizu, M., Yoshinaka, T., Hatori, S., & Kasai, H. (2005). Survivability of fish pathogenic viruses in environmental water, and inactivation of fish viruses. *Bulletin of Fisheries Research Agency Supplement, 2*, 47–54.
Yoshinaka, T., Yoshimizu, M., & Ezura, Y. (2000). Adsorption and infectivity of infectious hematopoietic necrosis virus (IHNV) with various solids. *Journal of Aquatic Animal Health, 12*, 64–66.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.