

TUMSAT-OACIS Repository - Tokyo

University of Marine Science and Technology

(東京海洋大学)

[1] Prevention and Treatment of Diseases Caused By Fish Pathogens

メタデータ	言語: eng 出版者: 公開日: 2016-05-13 キーワード (Ja): キーワード (En): 作成者: 吉水, 守, 笠井, 久会, 青木, 宙, 乙竹, 充, 酒井, 正博, Jung, Tae-Sung, 引間, 順一, 岡本, 信明, 坂本, 崇, 尾崎, 照遵, 矢澤, 良輔 メールアドレス: 所属:
URL	https://oacis.repo.nii.ac.jp/records/1266

aquaculture species. The generation of transgenic fish targeted on marine aquaculture species is still not popular due to the difficulties associated with handling small and fragile pelagic eggs. Recently, our group has developed a feasible and reproducible microinjection method for the pelagic eggs of marine fish and to establish stable transgenic strains in Nibe croaker, *Nibea mitsukurii* that could be a model species for the marine aquaculture fish species spawning pelagic eggs (Yamamoto et al, 2011). Accumulation of these techniques will realize the production of disease-resistant transgenic aquaculture species in near future.

3.2.4. Risks and Benefits of Transgenic Fish

Although, fish transgenesis has great advantages for the breeding of aquaculture species, there are several potential risks, particularly environmental and human health concerns. If transgenic fish escape into the natural environment, it would cause problems ecologically and genetically. Sterilization of transgenic strains with the polyploidy treatment and/or physical containment by the land-based marine aquaculture with the closed re-circulating system could be realistic way to solve this problem (Dunham, 2009). Another issue is human health concerns. To settle this issue, it is important to select the targeted gene, to conduct food safety trials securely and to keep consumers informed. Since it might be possible that transgenic fish with enhanced disease resistance may decrease or suppress the drug usage in aquaculture, this would improve the aquaculture production more safely from the standpoints of the drug residues and the emergence of antibiotic-resistant pathogens. Although it is essential to guarantee the safety of transgenic fish as genetically modified food, the disease-resistant transgenic fish could be of great help to improve the aquaculture.

Glossary

CMV: Cytomegalovirus

Bibliography

Section 1.1. Prevention

Yoshimizu M. (2003). Control Strategy for Viral Diseases of Salmonids and Flounder. In: *Biosecurity in Aquaculture Production Systems: Exclusion of Pathogens and Other Undesirables*, pp.35-41, Lee C.S. and Bryen, P.J.O' (eds.), World Aquaculture Society, Baton Rouge, LA, USA.

Yoshimizu M. (2009). Control Strategy for Viral Diseases of Salmonid Fish, Flounders and Shrimp at Hatchery and Seed Production Facility in Japan. *Fish Pathology* 44, 9-13.

Ahne W., Winton J.R. and Kimura T. (1989). Prevention of Infectious Diseases in Aquaculture. *Journal of Veterinary Medicine* 36, 561-567.

Kasai H., Muto Y. and Yoshimizu M. (2005). Virucidal Effects of Ultraviolet, Heat Treatment and Disinfectants against Koi Herpesvirus (KHV). *Fish Pathology* 40, 137-138.

Kasai H., Yoshimizu M. and Ezura Y. (2002). Disinfection of water for aquaculture. *Fisheries Science* 68 (Suppl.I), 821-824.

Yoshimizu M., Hyuga S., Oh M.-J., Ito S., Ezura Y. and Minura G. (1995). Disinfectant effect of oxidant produced by ozonation of sea water on fish pathogenic viruses, bacteria, and ciliate. In: *Diseases in Asian Aquaculture II*, pp.203-209, Shariff M., Arthur J.R. and Subasinghe R.P. (eds.), FHS/Asian Fisheries Society, Manila, Philippines.

Kasai H. and Yoshimizu M. (2002). Disinfection of water for aquaculture. In: Proceedings of the Workshop for FiSCUP - Health managements of aquatic organisms, pp.9-11, Cheju, Korea.

Yoshimizu M., Suzuki K., Nishizawa J., Winton R. and Exura Y. (1997). Antibody screening for the identification of nervous necrosis virus carriers in a flounder brood stock. In: New approaches to viral diseases of aquatic animals, pp.124-130, NRIA International Workshop, Mie, Japan..

Yoshimizu M., Kimura T. and Winton J.R. (1985). An improved technique for collecting reproductive fluid samples from salmonid fishes. *Progressive Fish-Culturist* 47, 199-200.

Yoshimizu M., Sami M. and Kimura T. (1989). Survivability of infectious haematopoietic necrosis virus in fertilized eggs of masu salmon and chum salmon. *Journal of Aquatic Animal Health* 1, 1-17.

Watanabe K., Nishizawa T. and Yoshimizu M. (2000). Selection of brood stock candidates of barfin flounder using an ELISA system with recombinant protein of barfin flounder nervous necrosis virus. *Disease of Aquatic Organisms* 41, 219-223.

Kohara K., Kasai H. and Yoshimizu M. (2012). Intra-ovum infection in salmonid eggs artificially contaminated with fish pathogenic bacteria: *Flavobacterium psychrophilum*, *Renibacterium salmoninarum* and *Aeromonas salmonicida*. *Fish Pathology* 47, 49-55.

Oseko N., Yoshimizu M. and Kimura T. (1992). Pathogenicity of Rhabdovirus olivaceus (hirame rhabdovirus; HRV) for salmonid fish. In: *Salmonid Diseases*, pp.80-87, Kimura T. (ed.), Hokkaido University Press, Sapporo, Japan.

Yoshimizu M. and Kimura T. (1976). Study on the Intestinal Microflora of Salmonids. *Fish Pathology* 10, 243-259.

Yoshimizu M., Fushimi Y., Kouno K., Shinada C., Ezura Y. and Kimura T. (1992). Biological Control of Infectious Hematopoietic Necrosis by Antiviral Substance Producing Bacteria. In: *Salmonid Diseases*, pp.301-307, Kimura T. (ed.), Hokkaido University Press, Sapporo, Japan.

Yoshimizu M. and Ezura Y. (2002). Biological control of fish viral disease with anti-viral substances produced by bacteria. In: Proceedings of The JSPS-NRCT International Symposium, pp.51-62, Rayong, Thailand.

Section: 1.2. Chemotherapy: Antimicrobial Agents for Aquaculture in Japan

Aoki, T. (1988) Drug-resistant plasmids from fish pathogens. *Microbiology Science*, 5, 219-223.

Aoki, T. (1992) Chemotherapy and drug resistance in fish farms in Japan; In *Diseases in Asian Aquaculture*, (Ed by M.Shriff, I., Subansinghe, R. P., Arthur J. R.), Fish Health Section, Asian Fisheries Society, 519-529 pp. Manila, Philippines.

Aoki, T., Satoh, T., Kitao, T. (1987) New tetracycline resistance determinant on R plasmids from *Vibrio anguillarum*. *Antimicrobial Agents and Chemotherapy*, 31, 1446-1449.

del Castillo, C. S., Hikima, J., Jang, H. B., Nho, S. W., Jung, T. S., Wongtavatchai, J., Kondo, H., Hirono, I., Takeyama, H. and Aoki, T. (2013) Comparative sequence analysis of a multi-drug resistant plasmid from *Aeromonas hydrophila*. *Antimicrobial Agents and Chemotherapy*, 57, 120-129.

Han, J. E., Kim, J. H., Choresca Jr, C. H., Shin, S. P., Jun, J. W., Chai, J. Y. and Park, S. C. (2012a) A small IncQ-type plasmid carrying the quinolone resistance (*qnrS2*) gene from *Aeromonas hydrophila*. *Letters in Applied Microbiology*, 54, 374-376.

Han, J. E., Kim, J. H., Choresca Jr, C. H., Shin, S. P., Jun, J. W., Chai, J. Y. and Park, S. C. (2012b) First description of ColE type plasmid in *Aeromonas* spp. carrying quinolone resistance (*qnrS2*) gene. *Letters in Applied Microbiology*, 55, 290-294.

Han, J. E., Kim, J. H., Choresca, C. H., Jr, Shin, S. P., Jun, J. W., Chai, J. Y., Han, S. Y. and Park, S. C. (2012c) First description of the *qnrS*-like (*qnrS5*) gene and analysis of quinolone resistance-determining regions in motile *Aeromonas* spp. from diseased fish and water. *Research Microbiology*, 163, 73-79.

Kim, M. J., Hirono, I., Kurokawa, K., Maki, T., Hawke, J., Kondo, H., Santos, M. D. and Aoki, T. (2008), Complete DNA sequence and analysis of the transferable multiple-drug resistance plasmids (R plasmids)

from *Photobacterium damsela* subsp. *piscicida* isolates collected in Japan and the United States. *Antimicrobial Agents and Chemotherapy*, 52, 606-611.

Maki, T., Santos, M. D., Kondo, H., Hirono, I. and Aoki, T. (2009) A transferable 20-kilobase multiple drug resistance-conferring R plasmid (pKL0018) from a fish pathogen (*Lactococcus garvieae*) is highly homologous to a conjugative multiple drug resistance-conferring enterococcal plasmid. *Applied and Environmental Microbiology*, 75, 3370-3372.

Miller, RA, Walker, R. D., Carson, J., Coles, M., Coyne, R., Dalsgaard, I., Gieseke, C., Hsu, H. M., Mathers, J. J., Papapetropoulou, M., Petty, B., Teitzel, C. and Reimschuessel, R. (2005) Standardization of a broth microdilution susceptibility testing method to determine minimum inhibitory concentrations of aquatic bacteria. *Diseases of Aquatic Organisms*, 64, 211-222.

Revised Standard Method of the Japanese Society of Antimicrobials for Animals in 2003 (2003) The determination method of minimal inhibitory concentration (MIC) of antimicrobials against bacteria isolated from animals. *Proceeding of the Japanese of Antimicrobials for Animals*, 25, 63-73.

Rodkhum, C., Maki, T., Hirono, I. and Aoki, T. (2008) *gyrA* and *parC* associated with quinolone resistance in *Vibrio anguillarum*. *Journal of Fish Diseases*, 31, 395-399.

Sørum, H. (2006), Antimicrobial drug resistance in fish pathogens. Chapter 13, pp. 213-238, In Aarestrup FM (ed.), *Antimicrobial Resistance in Bacteria of Animal Origin*, ASM Press, Washington D.C.

Welch, T. J., Evenhuis, J., White, D. G., McDermott, P. F., Harbottle, H., Miller, R. A., Griffin, M. and Wise, D. (2009) IncA/C plasmid-mediated florfenicol resistance in the catfish pathogen *Edwardsiella ictaluri*. *Antimicrobial Agents and Chemotherapy*, 53, 845-846.

del Castillo, C. S., Jang, H. B., Hikima, J., Jung, T. S., Morii, H., Hirono, I., Kondo, H., Kurosaka, C. and Aoki, T. (2013) Comparative analysis and distribution of pP9014, a novel drug resistance IncP-1 plasmid from *Photobacterium damsela* subsp. *piscicida*. *International Journal of Antimicrobial Agents*, 42, 10-18.

Section: 1.3. Vaccination – Injection, Oral and Immersion

Iida T., Wakabayashi H. and Egusa S. (1982). Vaccination for control of streptococcal disease in cultured yellowtail. *Fish Pathology* 16, 201-206.

Kai Y.H. and Chi S.C. (2008). Efficacies of inactivated vaccines against betanodavirus in grouper larvae (*Epinephelus coioides*) by bath immunization. *Vaccine* 26, 1450-1457.

Nakanishi T. and Ototake M. (1997). Antigen uptake and immune responses after immersion vaccination. In: *Fish vaccinology. Developments in biological standardization*, Vol 90, pp.59-68, Gudding R., Lillehaug A., Midtlyng P.J. and Brown F. (eds.), Karger, Basel, Switzerland.

Gould R.W., O'Leary P.J., Garrison R.L., Rohovec J.S. and Fryer J.L. (1978). Spray Vaccination: a method for the immunization of fish. *Fish Pathology* 13, 63-68.

Zhou Y.C., Huang H., Wang J., Zhang B. and Su Y.Q. (2002). Vaccination of the grouper, *Epinephalus awoara*, against vibriosis using the ultrasonic technique. *Aquaculture*, 203, 229-238.

Nakanishi T., Kiryu I. and Ototake M. (2002). Development of a new vaccine delivery method for fish: percutaneous administration by immersion with application of a multiple puncture instrument. *Vaccine* 20, 3764-3769.

Fender D.C. and Amend D.F. (1978). Hyperosmotic infiltration: factors influencing uptake of bovine serum albumin by rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* 35, 871-874.

Thune R.L. and Plumb J.A. (1984). Evaluation of hyperosmotic infiltration for the administration of antigen to channel catfish (*Ictalurus punctatus*). *Aquaculture* 36, 1-8.

Ototake M. and Nakanishi T. (1992a). Kinetics of bovine serum albumin in fish plasma after hyperosmotic infiltration treatment: comparison between marine and freshwater fish. *Aquaculture* 103, 229-240.

Ototake M. and Nakanishi T. (1992b). Effects of water temperature on kinetics of bovine serum albumin in the plasma of rainbow trout *Oncorhynchus mykiss* after bath administration. *Nippon Suisan Gakkaishi* 58, 1301-1305.

Kiryu I., Ootake M., Nakanishi T. and Wakabayashi H. (2000). The uptake of fluorescent microspheres into the skin, fins and gills of rainbow trout during immersion. *Fish Pathology* 35, 41-48.

dos Santos N.M.S., Taverne-Thiele J.J., Barnes T.T.A.C., Muiswinkel W.B.V., Ellis A.E. and Rombout J.H.W.M. (2001). The gill is a major organ for antibody secreting cell production following direct immersion of sea bass (*Dicentrarchus labrax*, L.) in a *Photobacterium damsela* ssp. *piscicida* bacterin: an ontogenetic study. *Fish and Shellfish Immunology* 11, 65-74.

Swan C.M., Lindstrom N.M. and Cain K.D. (2008). Identification of a localized mucosal immune response in rainbow trout, *Oncorhynchus mykiss* (Walbaum), following immunization with a protein-hapten antigen. *Journal of Fish Diseases* 31, 383-393.

Vervarcke S., Ollevier F., Kinget R. and Michoel A. (2005). Mucosal response in African catfish after administration of *Vibrio anguillarum* O2 antigens via different routes. *Fish and Shellfish Immunology* 18, 125-133.

Esteve-Gassent M.D., Nielsen M.E. and Amaro C. (2003). The kinetics of antibody production in mucus and serum of European eel (*Anguilla anguilla* L.) after vaccination against *Vibrio vulnificus*: development of a new method for antibody quantification in skin mucus. *Fish and Shellfish Immunology* 15, 51-61.

Section: 1.4. Vaccination – Recombinant and DNA Vaccines

Acosta F., Petrie A., Lockhart K., Lorenzen N. and Ellis A.E. (2005). Kinetics of Mx expression in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar* L.) parr in response to VHS-DNA vaccination. *Fish and Shellfish Immunology* 18, 81-89.

Alcorn S.W. and Pascho R.J. (2000). Single-dilution enzyme-linked immunosorbent assay for quantification of antigen-specific salmonid antibody. *Journal of Veterinary Diagnostic Investigation* 12, 245-252.

Allnutt F.C., Bowers R.M., Rowe C.G., Vakharia V.N., LaPatra S.E. and Dhar A.K. (2007). Antigenicity of infectious pancreatic necrosis virus VP2 subviral particles expressed in yeast. *Vaccine* 25, 4880-4888.

Alonso M., Chiou P.P. and Leong J.A. (2011). Development of a suicidal DNA vaccine for infectious hematopoietic necrosis virus (IHNV). *Fish and Shellfish Immunology* 30, 815-823.

Álvarez B., Álvarez J., Menéndez A. and Guijarro J.A. (2008). A mutant in one of two *exbD* loci of a TonB system in *Flavobacterium psychrophilum* shows attenuated virulence and confers protection against cold water disease. *Microbiology* 154, 1144-1151.

Anderson E.D., Mourich D.V., Fahrenkrug S.C., LaPatra S., Shepherd J. and Leong J.A. (1996). Genetic immunization of rainbow trout (*Oncorhynchus mykiss*) against infectious hematopoietic necrosis virus. *Molecular marine biology and biotechnology* 5, 114-122.

Biacchesi S., Thoulouze M.I., Béarzotti M., Yu Y.X. and Brémont M. (2000). Recovery of NV knockout infectious hematopoietic necrosis virus expressing foreign genes. *Journal of Virology* 74, 11247-11253.

Biacchesi S., Béarzotti M., Bouguyon E. and Brémont M. (2002). Heterologous exchanges of the glycoprotein and the matrix protein in a Novirhabdovirus. *Journal of Virology* 76, 2881-2889.

Boudinot P., Bernard D., Boubekeur S., Thoulouze M.I., Brémont M. and Benmansour A. (2004). The glycoprotein of a fish rhabdovirus profiles the virus-specific T-cell repertoire in rainbow trout. *Journal of General Virology* 85, 3099-3108.

Boudinot P., Blanco M., de Kinkelin P. and Benmansour A. (1998). Combined DNA immunization with the glycoprotein gene of viral hemorrhagic septicemia virus and infectious hematopoietic necrosis virus induces double-specific protective immunity and nonspecific response in rainbow trout. *Virology* 249(2), 297-306.

Buchanan J.T., Stannard J.A., Lauth X., Ostland V.E., Powell H.C., Westerman M.E. and Nizet V. (2005). *Streptococcus iniae* phosphoglucosyltransferase is a virulence factor and a target for vaccine development. *Infection and Immunity* 73, 6935-6944.

Byon J.Y., Ohira T., Hirono I. and Aoki T. (2005). Use of a cDNA microarray to study immunity against viral hemorrhagic septicemia (VHS) in Japanese flounder (*Paralichthys olivaceus*) following DNA vaccination. *Fish and Shellfish Immunology* 18(2), 135-147.

Byon J.Y., Ohira T., Hirono I. and Aoki T. (2006). Comparative immune responses in Japanese flounder, *Paralichthys olivaceus* after vaccination with viral hemorrhagic septicemia virus (VHSV) recombinant glycoprotein and DNA vaccine using a microarray analysis. *Vaccine* 24(7), 921-930.

Cain K.D., LaPatra S.E., Shewmaker B., Jones J., Byrne K.M. and Ristow S.S. (1999a). Immunogenicity of a recombinant infectious hematopoietic necrosis virus glycoprotein produced in insect cells. *Diseases of Aquatic Organisms* 36, 67-72.

Cain K.D., Byrne K.M., Brassfield A.L., LaPatra S.E. and Ristow S.S. (1999b). Temperature dependent characteristics of a recombinant infectious hematopoietic necrosis virus glycoprotein produced in insect cells. *Diseases of Aquatic Organisms* 36, 1-10.

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

Caipang C.M., Takano T., Hirono I. and Aoki T. (2006b). Genetic vaccines protect red seabream, *Pagrus major*, upon challenge with red seabream iridovirus (RSIV). *Fish and Shellfish Immunology* 21(2), 130-138.

Carpio Y., Basabe L., Acosta J., Rodríguez A., Mendoza A., Lisperger A., Zamorano E., González M., Rivas M., Contreras S., Haussmann D., Figueroa J., Osorio, V.N., Asencio G., Mancilla J., Ritchie G., Borroto C. and Estrada M.P. (2011). Novel gene isolated from *Caligus rogercresseyi*: a promising target for vaccine development against sea lice. *Vaccine* 29, 2810-2820.

Cheng S., Hu Y.H., Jiao X.D. and Sun L. (2010). Identification and immunoprotective analysis of a *Streptococcus iniae* subunit vaccine candidate. *Vaccine* 28, 2636-2641.

Chico V., Ortega-Villaizan M., Falco A., Tafalla C., Perez L., Coll J.M. and Estepa A. (2009). The immunogenicity of viral haemorrhagic septicaemia rhabdovirus (VHSV) DNA vaccines can depend on plasmid regulatory sequences. *Vaccine* 27(13), 1938-1948.

Choi S.H. and Kim K.H. (2011). Generation of two auxotrophic genes knock-out *Edwardsiella tarda* and assessment of its potential as a combined vaccine in olive flounder (*Paralichthys olivaceus*). *Fish and Shellfish Immunology* 31, 58-65.

Christie K.E. (1997). Immunization with viral antigens: infectious pancreatic necrosis. In "Fish Vaccinology" (ed. by R. Gudding, A. Lillehaug, P. J. Midtlyng and F. Brown). *Developments in Biological Standardization* (Basel), Switzerland, Karger, vol. 90, p191-199.

Clark T.G., Gao Y., Gaertig J., Wang X. and Cheng G. (2001). The I-antigens of *Ichthyophthirius multifiliis* are GPI-anchored proteins. *Journal of Eukaryotic Microbiology* 48, 332-337.

Corbeil S., Kurath G. and LaPatra S.E. (2000a). Fish DNA vaccine against infectious hematopoietic necrosis virus: efficacy of various routes of immunisation. *Fish and Shellfish Immunology* 10, 711-723.

Corbeil S., LaPatra S.E., Anderson E.D. and Kurath G. (2000b). Nanogram quantities of a DNA vaccine protect rainbow trout fry against heterologous strains of infectious hematopoietic necrosis virus. *Vaccine* 18, 2817-2824.

Corbeil S., LaPatra S.E., Anderson E.D., Jones J., Vincent B., Hsu Y.L. and Kurath G. (1999). Evaluation of the protective immunogenicity of the N, P, M, NV and G proteins of infectious hematopoietic necrosis virus in rainbow trout *Oncorhynchus mykiss* using DNA vaccines. *Diseases of Aquatic Organisms* 39, 29-36.

Costes B., Fournier G., Michel B., Delforge C., Raj V.S., Dewals B., Gillet L., Drion P., Body A., Schynts F., Lieffrig F. and Vanderplasschen A. (2008). Cloning of the koi herpesvirus genome as an infectious bacterial artificial chromosome demonstrates that disruption of the thymidine kinase locus induces partial attenuation in *Cyprinus carpio* koi. *Journal of Virology* 82, 4955-4964.

Cuesta A. and Tafalla C. (2009). Transcription of immune genes upon challenge with viral hemorrhagic septicemia virus (VHSV) in DNA vaccinated rainbow trout (*Oncorhynchus mykiss*). *Vaccine* 27(2), 280-289.

Dang, W., Zhang M. and Sun L. (2011). *Edwardsiella tarda* DnaJ is a virulence-associated molecular chaperone with immunoprotective potential. *Fish and Shellfish Immunology* 31, 182-188.

de las Heras A.I., Rodríguez Saint-Jean S. and Pérez-Prieto S.I. (2010). Immunogenic and protective effects of an oral DNA vaccine against infectious pancreatic necrosis virus in fish. *Fish and Shellfish Immunology* 28, 562-570.

Dijkstra J.M., Fischer U., Sawamoto Y., Ootake M. and Nakanishi T. (2001). Exogenous antigens and the stimulation of MHC class I restricted cell-mediated cytotoxicity: possible strategies for fish vaccines. *Fish and Shellfish Immunology* 11, 437-458.

Dorson M. (1988). Vaccination against infectious pancreatic necrosis. In "Fish vaccination" (ed. by Ellis A.E.), Academic Press. London, UK, p162-171.

Emmenegger E.J. and Kurath G. (2008). DNA vaccine protects ornamental koi (*Cyprinus carpio koi*) against North American spring viremia of carp virus. *Vaccine* 26, 6415-6521.

Engelking H.M. and Leong J.C. (1989a). Glycoprotein from infectious hematopoietic necrosis virus (IHNV) induces protective immunity against five IHNV types. *Journal of Aquatic Animal Health* 1, 291-300.

Engelking H.M. and Leong J.C. (1989b). The glycoprotein of infectious hematopoietic necrosis virus elicits neutralizing antibody and protective responses. *Virus Research* 13, 213-230.

Estepa A. and Coll J.M. (1993). Enhancement of fish mortality by rhabdovirus infection after immunization with a viral nucleoprotein peptide. *Viral Immunology* 6, 237-243.

Estepa A., Thiry M. and Coll J.M. (1994). Recombinant protein fragments from haemorrhagic septicaemia rhabdovirus stimulate trout leukocyte anamnestic responses *in vitro*. *Journal of General Virology* 75, 1329-1338.

Fernandez-Alonso M., Rocha A. and Coll J.M. (2001). DNA vaccination by immersion and ultrasound to trout viral haemorrhagic septicaemia virus. *Vaccine* 19(23-24), 3067-3075.

Garver K.A., LaPatra S.E. and Kurath G. (2005). Efficacy of an infectious hematopoietic necrosis (IHNV) virus DNA vaccine in Chinook *Oncorhynchus tshawytscha* and sockeye *O. nerka* salmon. *Diseases of Aquatic Organisms* 64, 13-22.

Gillund F., Dalmo R., Tonheim T.C., Seternes T. and Myhr A.I. (2008a). DNA vaccination in aquaculture – Expert judgments of impacts on environment and fish health. *Aquaculture*, 284, 25-34.

Gillund F., Kjåberg K.A., von Krauss M.K. and Myhr A.I. (2008b). Do uncertainty analyses reveal uncertainties? Using the introduction of DNA vaccines to aquaculture as a case. *Science of the Total Environment* 407, 185-196

Gilmore Jr. R.D., Engelking H.M., Manning D.S. and Leong J.C. (1988). Expression in *Escherichia coli* of an epitope of the glycoprotein of infectious hematopoietic necrosis virus protects against viral challenge. *Bio/Technology* 6, 295-300.

Grayson T.H., Cooper L.F., Wrathmell A.B., Roper J., Evenden A.J. and Gilpin M.L. (2002). Host responses to *Renibacterium salmoninarum* and specific components of the pathogen reveal the mechanisms of immune suppression and activation. *Immunology* 106, 273-283.

Gómez-Casado E., Estepa A. and Coll J.M. (2011). A comparative review on European-farmed finfish RNA viruses and their vaccines. *Vaccine* 29, 2657-2671.

Grøntvedt R.N. and Espelid S. (2004). Vaccination and immune responses against atypical *Aeromonas salmonicida* in spotted wolffish (*Anarhichas minor* Olafsen) juveniles. *Fish and Shellfish Immunology* 16, 271-285.

Grove S., Høie S. and Evensen Ø. (2003). Distribution and retention of antigens of *Aeromonas salmonicida* in Atlantic salmon (*Salmo salar* L.) vaccinated with a Δ aroA mutant or formalin-inactivated bacteria in oil-adjuvant. *Fish and Shellfish Immunology* 15, 349-358.

Guan L., Mu W., Champeimont J., Wang Q., Wu H., Xiao J., Lubitz W., Zhang Y. and Liu Q. (2011a). Iron-regulated lysis of recombinant *Escherichia coli* in host releases protective antigen and confers biological containment. *Infection and Immunity* 79, 2608-2618.

Guan R., Xiong J., Huang W. and Guo S. (2011b). Enhancement of protective immunity in European eel

(*Anguilla anguilla*) against *Aeromonas hydrophila* and *Aeromonas sobria* by a recombinant *Aeromonas* outer membrane protein. *Acta Biochimica et Biophysica Sinica* (Shanghai) 43, 79-88.

He J., Yin Z., Xu G., Gong Z., Lam T.J. and Sin Y.M. (1997). Protection of goldfish against *Ichthyophthirius multifiliis* by immunization with a recombinant vaccine. *Aquaculture* 158, 1-10.

Heppell J., Lorenzen N., Armstrong N.K., Wu T., Lorenzen E., Einer-Jensen K., Schorr J. and Davis H.L. (1998). Development of DNA vaccines for fish: vector design, intramuscular injection and antigen expression using viral haemorrhagic septicaemia virus genes as model. *Fish and Shellfish Immunology* 8(4), 271-286.

Ho L.P., Han-You Lin J., Liu H.C., Chen H.E., Chen T.Y. and Yang H.L. (2011). Identification of antigens for the development of a subunit vaccine against *Photobacterium damsela* ssp. *piscicida*. *Fish and Shellfish Immunology* 30, 412-419.

Hu Y.H., Dang W., Deng T. and Sun L. (2012). *Edwardsiella tarda* DnaK: expression, activity, and the basis for the construction of a bivalent live vaccine against *E. tarda* and *Streptococcus iniae*. *Fish and Shellfish Immunology* 32, 616-620.

Hu Y.H. and Sun L. (2011). A bivalent *Vibrio harveyi* DNA vaccine induces strong protection in Japanese flounder (*Paralichthys olivaceus*). *Vaccine* 29, 4328-4333.

Húsgað S., Grotmol S., Hjeltnes B.K., Rødseth O.M. and Biering E. (2001). Immune response to a recombinant capsid protein of striped jack nervous necrosis virus (SJNNV) in turbot *Scophthalmus maximus* and Atlantic halibut *Hippoglossus hippoglossus*, and evaluation of a vaccine against SJNNV. *Diseases of Aquatic Organisms* 45, 33-44.

Jiao X.D., Zhang M., Hu Y.H. and Sun L. (2009). Construction and evaluation of DNA vaccines encoding *Edwardsiella tarda* antigens. *Vaccine* 27, 5195-5202.

Jiao X.D., Zhang M., Cheng S. and Sun L. (2010). Analysis of *Edwardsiella tarda* DegP, a serine protease and a protective immunogen. *Fish and Shellfish Immunology* 28, 672-677.

Kanellos T., Sylvester I.D., D'Mello F., Howard C.R., Mackie A., Dixon P.F., Chang K.-C., Ramstad A., Midtlyng P.J. and Russell P.H. (2006). DNA vaccination can protect *Cyprinus carpio* against spring viraemia of carp virus. *Vaccine* 24, 4927-4933.

Khushiramani R.M., Maiti B., Shekar M., Girisha S.K., Akash N., Deepanjali A., Karunasagar I. and Karunasagar I. (2012). Recombinant *Aeromonas hydrophila* outer membrane protein 48 (Omp48) induces a protective immune response against *Aeromonas hydrophila* and *Edwardsiella tarda*. *Research in Microbiology* 163, 286-291.

Kim C.H., Johnson M.C., Drennan J.D., Simon B.E., Thomann E. and Leong J.A. (2000). DNA vaccines encoding viral glycoproteins induce nonspecific immunity and Mx protein synthesis in fish. *Journal of Virology* 74, 7048-7054.

Kim M.S., Kim D.S. and Kim K.H. (2011). Oral immunization of olive flounder (*Paralichthys olivaceus*) with recombinant live viral hemorrhagic septicemia virus (VHSV) induces protection against VHSV infection. *Fish and Shellfish Immunology* 31, 212-216.

Kim T.J., Jang E.J. and Lee J.I. (2008). Vaccination of rock bream, *Oplegnathus fasciatus* (Temminck & Schlegel), using a recombinant major capsid protein of fish iridovirus. *Journal of Fish Diseases* 31, 547-551.

Kumar S.R., Parameswaran V., Ahmed V.P., Musthaq S.S. and Hameed A.S. (2007). Protective efficiency of DNA vaccination in Asian seabass (*Lates calcarifer*) against *Vibrio anguillarum*. *Fish and Shellfish Immunology* 23, 316-326.

Kumar S.R., Ahmed V.P.I., Parameswaran V., Sudhakaran R., Babu V.S. and Hameed A.S.S. (2008a). Potential use of chitosan nanoparticle for oral delivery of DNA vaccine in Asian sea bass (*Lates calcarifer*) to protect from *Vibrio* (*Listonella*) *anguillarum*. *Fish and Shellfish Immunology* 25, 47-56.

Kumar S.R., Ahmed V.P.I., Sarathi M., Basha A.N. and Hameed A.S.S. (2008b). Immunological response of *Penaeus monodon* to DNA vaccine and its efficacy to protect shrimp against white spot syndrome virus (WSSV). *Fish and Shellfish Immunology* 24, 467-478.

Kurath G. (2008). Biotechnology and DNA vaccines for aquatic animals. Scientific and Technical Review of the Office *International des Epizooties* 27, 175-196.

Kuzyk M.A., Burian J., Machander D., Dolhaine D., Cameron S., Thornton J.C. and Kay W.W. (2001a). An efficacious recombinant subunit vaccine against the salmonid rickettsial pathogen *Piscirickettsia salmonis*. *Vaccine* 19, 2337-2344.

Kuzyk M.A., Burian J., Thornton J.C. and Kay W.W. (2001b). OspA, a lipoprotein antigen of the obligate intracellular bacterial pathogen *Piscirickettsia salmonis*. *Journal of Molecular Microbiology and Biotechnology* 3, 83-93.

Lan, M.Z., Peng X., Xiang M.Y., Xia Z.Y., Bo W., Jie L., Li X.Y. and Jun Z.P. (2007). Construction and characterization of a live, attenuated *esrB* mutant of *Edwardsiella tarda* and its potential as a vaccine against the haemorrhagic septicaemia in turbot, *Scophthamus maximus* (L.). *Fish and Shellfish Immunology* 23, 521-530.

LaPatra S.E., Corbeil S., Jones G.R., Shewmaker W.D., Lorenzen N., Anderson E.D. and Kurath G. (2001). Protection of rainbow trout against infectious hematopoietic necrosis virus four days after specific or semi-specific DNA vaccination. *Vaccine* 19, 4011-4019.

Lawrence M.L., Cooper R.K. and Thune R.L. (1997). Attenuation, persistence, and vaccine potential of an *Edwardsiella ictaluri* *purA* mutant. *Infection and Immunity* 65, 4642-4651.

Lecocq-Xhonneux F., Thiry M., Dheur I., Rossius M., Vanderheijden N., Martial J. and de Kinkelin P. (1994). A recombinant viral haemorrhagic septicaemia virus glycoprotein expressed in insect cells induces protective immunity in rainbow trout. *Journal of General Virology* 75, 1579-1587.

Leong J.A. (1993). Molecular and biotechnological approaches to fish vaccines. *Current Opinion in Biotechnology* 4, 286-293.

Leong J.C., Barrie R., Engelking H.M., Feyereisen-Koener J., Gilmore R., Harry J., Kurath G., Manning D.S., Mason C.L., Oberg L. and Wirkkula J. (1987) Recombinant viral vaccines in aquaculture. In "Genetics in aquaculture: Proceedings of the sixteenth U.S.-Japan meeting on aquaculture" (ed. by Svrjcek R.S.), NOAA Technical Report NMFS, 92, p107-111.

Liang H.Y., Wu Z.H., Jian J.C. and Huang Y.C. (2011). Protection of red snapper (*Lutjanus sanguineus*) against *Vibrio alginolyticus* with a DNA vaccine containing flagellin *flaA* gene. *Letters in Applied Microbiology* 52, 156-161.

Li M.F., Hu Y.H., Zheng W.J., Sun B.G., Wang C.L. and Sun L. (2012). *Inv1*: an *Edwardsiella tarda* invasin and a protective immunogen that is required for host infection. *Fish and Shellfish Immunology* 32, 586-592.

Liu R., Chen J., Li K. and Zhang X. (2011). Identification and evaluation as a DNA vaccine candidate of a virulence-associated serine protease from a pathogenic *Vibrio parahaemolyticus* isolate. *Fish and Shellfish Immunology* 30, 1241-1248.

Liu Y., Oshima S., Kurohara K., Ohnishi K. and Kawai K. (2005). Vaccine efficacy of recombinant GAPDH of *Edwardsiella tarda* against Edwardsiellosis. *Microbiology and Immunology* 49, 605-612.

Liu W., Hsu C.H., Chang C.Y., Chen H.H. and Lin C.S. (2006). Immune response against grouper nervous necrosis virus by vaccination of virus-like particles. *Vaccine* 24, 6282-6287.

Locke J.B., Aziz R.K., Vicknair M.R., Nizet V. and Buchanan J.T. (2008). *Streptococcus iniae* M-like protein contributes to virulence in fish and is a target for live attenuated vaccine development. *PLoS One* 3, e2824.

Lorenzen N., Lorenzen E., Einer-Jensen K., Heppell J., Wu T. and Davis H. (1998). Protective immunity to VHS in rainbow trout (*Oncorhynchus mykiss*, Walbaum) following DNA vaccination. *Fish and Shellfish Immunology* 8(4), 261-270.

Lorenzen N., Olesen N.J., Jørgensen P.E., Etzerodt M., Holtet T.L. and Thøgersen H.C. (1993). Molecular cloning and expression in *Escherichia coli* of the glycoprotein gene of VHS virus, and immunization of rainbow trout with the recombinant protein. *Journal of General Virology* 74, 623-630.

Lorenzen N. and Olesen N.J. (1997). Immunization with viral antigens: viral haemorrhagic septicaemia. In

“Fish Vaccinology” (ed. by R. Gudding, A. Lillehaug, P. J. Midtlyng and F. Brown). *Dev. Biol Stand.* Basel, Switzerland, Karger, vol. 90, p201-209.

Lorenzo G.A., Estepa A., Chilmonczyk S. and Coll J.M. (1995). Different peptides from hemorrhagic septicemia rhabdoviral proteins stimulate leucocyte proliferation with individual fish variation. *Virology* 212, 348-355.

Lund V., Espelid S. and Mikkelsen H. (2003). Vaccine efficacy in spotted wolffish *Anarhichas minor*: relationship to molecular variation in A-layer protein of atypical *Aeromonas salmonicida*. *Diseases of Aquatic Organisms* 56, 31-42.

Maiti B., Shetty M., Shekar M., Karunasagar I. and Karunasagar I. (2011). Recombinant outer membrane protein A (OmpA) of *Edwardsiella tarda*, a potential vaccine candidate for fish, common carp. *Microbiological Research* 167, 1-7.

Marsden M.J., Vaughan L.M., Foster T.J. and Secombes C.J. (1996). A live (delta aroA) *Aeromonas salmonicida* vaccine for furunculosis preferentially stimulates T-cell responses relative to B-cell responses in rainbow trout (*Oncorhynchus mykiss*). *Infection and Immunity* 64, 3863-3869.

Marshall S.H., Conejeros P., Zahr M., Olivares J., Gómez F., Cataldo P. and Henríquez V. (2007). Immunological characterization of a bacterial protein isolated from salmonid fish naturally infected with *Piscirickettsia salmonis*. *Vaccine* 25, 2095-2102.

Martin S.A., Blaney S.C., Houlihan D.F. and Secombes C.J. (2006). Transcriptome response following administration of a live bacterial vaccine in Atlantic salmon (*Salmo salar*). *Molecular Immunology* 43, 1900-1911.

Maurice S., Dekel M., Shoseyov O. and Gertler A. (2003). Cellulose beads bound to cellulose binding domain-fused recombinant proteins; an adjuvant system for parenteral vaccination of fish. *Vaccine* 21, 3200-3207.

Maurice S., Nussinovitch A., Jaffe N., Shoseyov O. and Gertler A. (2004). Oral immunization of *Carassius auratus* with modified recombinant A-layer proteins entrapped in alginate beads. *Vaccine* 23, 450-459.

Mikalsen A.B., Sindre H., Torgersen J. and Rimstad E. (2005). Protective effects of a DNA vaccine expressing the infectious salmon anemia virus hemagglutinin-esterase in Atlantic salmon. *Vaccine* 23, 4895-4905.

Mikalsen A.B., Torgersen J., Aleström P., Helleman A.L., Koppang E.O. and Rimstad E. (2004). Protection of atlantic salmon *Salmo salar* against infectious pancreatic necrosis after DNA vaccination. *Diseases of Aquatic Organisms* 60, 11-20.

Min L., Li-Li Z., Jun-Wei G., Xin-Yuan Q., Yi-Jing L. and Di-Qiu L. (2012). Immunogenicity of *Lactobacillus*-expressing VP2 and VP3 of the infectious pancreatic necrosis virus (IPNV) in rainbow trout. *Fish and Shellfish Immunology* 32, 196-203.

Miquel A., Müller I., Ferrer P., Valenzuela P.D. and Burzio L.O. (2003). Immunoresponse of Coho salmon immunized with a gene expression library from *Piscirickettsia salmonis*. *Biological Research* 36, 313-323.

Moral C.H., del Castillo E.F., Fierro P.L., Cortés A.V., Castillo J.A., Soriano A.C., Salazar M.S., Peralta B.R. and Carrasco G.N. (1998). Molecular characterization of the *Aeromonas hydrophila* aroA gene and potential use of an auxotrophic aroA mutant as a live attenuated vaccine. *Infection and Immunity* 66, 1813-1821.

Mu W., Guan L., Yan Y., Liu Q. and Zhang Y. (2011a). A novel in vivo inducible expression system in *Edwardsiella tarda* for potential application in bacterial polyvalence vaccine. *Fish and Shellfish Immunology* 31, 1097-1105.

Myhr A.I. and Dalmo R.A. (2005). Introduction of genetic engineering in aquaculture: Ecological and ethical implications for science and governance. *Aquaculture* 250, 542-554.

Ningqiu L., Junjie B., Shuqin W., Xiaozhe F., Haihua L., Xing Y. and Cunbin S. (2008). An outer membrane protein, *OmpK*, is an effective vaccine candidate for *Vibrio harveyi* in Orange-spotted grouper (*Epinephelus coioides*). *Fish and Shellfish Immunology* 25, 829-833.

Noonan B., Enzmann P.J. and Trust T.J. (1995). Recombinant infectious hematopoietic necrosis virus and

viral hemorrhagic septicemia virus glycoprotein epitopes expressed in *Aeromonas salmonicida* induce protective immunity in rainbow trout (*Oncorhynchus mykiss*). *Applied and Environmental Microbiology* 61, 3586-3591.

Novoa B., Romero A., Mulero V., Rodríguez I., Fernández I. and Figueras A. (2006). Zebrafish (*Danio rerio*) as a model for the study of vaccination against viral haemorrhagic septicemia virus (VHSV). *Vaccine* 24, 5806-5816.

Nusbaum K.E., Smith B.F., DeInnocentes P. and Bird R.C. (2002). Protective immunity induced by DNA vaccination of channel catfish with early and late transcripts of the channel catfish herpesvirus (IHV-1). *Veterinary Immunology and Immunopathology* 84, 151-168.

Oberg L.A., Wirkkula J., Mourich D. and Leong J.C. (1991). Bacterially expressed nucleoprotein of infectious hematopoietic necrosis virus augments protective immunity induced by the glycoprotein vaccine in fish. *Journal of Virology* 65, 4486-4489.

Pan C.Y., Huang T.C., Wang Y.D., Yeh Y.C., Hui C.F. and Chen J.Y. (2012). Oral administration of recombinant epinecidin-1 protected grouper (*Epinephelus coioides*) and zebrafish (*Danio rerio*) from *Vibrio vulnificus* infection and enhanced immune-related gene expressions. *Fish and Shellfish Immunology* 32, 947-957.

Pasnik D.J. and Smith S.A. (2005). Immunogenic and protective effects of a DNA vaccine for *Mycobacterium marinum* in fish. *Veterinary Immunology and Immunopathology* 103, 195-206.

Pasnik D.J. and Smith S.A. (2006). Immune and histopathologic responses of DNA-vaccinated hybrid striped bass *Morone saxatilis* x *M. chrysops* after acute *Mycobacterium marinum* infection. *Diseases of Aquatic Organisms* 73, 33-41.

Priya T.A.J., Lin Y.-H., Wang Y.-C., Yang C.-S., Chang P.-S. and Song Y.-L. (2012). Codon changed immobilization antigen (iAg), a potent DNA vaccine in fish against *Cryptocaryon irritans* infection. *Vaccine* 30, 893-903.

Qian R., Chu W., Mao Z., Zhang C., Wei Y. and Yu L. (2007). Expression, characterization and immunogenicity of a major outer membrane protein from *Vibrio alginolyticus*. *Acta Biochimica et Biophysica Sinica* (Shanghai) 39, 194-200.

Ramstad A., Romstad A.B., Knappskog D.H. and Midtlyng P.J. (2007). Field validation of experimental challenge models for IPN vaccines. *Journal of Fish Diseases* 30, 723-731.

Romero A., Figueras A., Tafalla C., Thoulouze M.I., Bremont M. and Novoa B. (2005). Histological, serological and virulence studies on rainbow trout experimentally infected with recombinant infectious hematopoietic necrosis viruses. *Diseases of Aquatic Organisms* 68, 17-28.

Romero A., Figueras A., Thoulouze M.I., Bremont M. and Novoa B. (2008). Recombinant infectious hematopoietic necrosis viruses induce protection for rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms* 80, 123-135.

Romero A., Dios S., Bremont M., Figueras A. and Novoa B. (2011). Interaction of the attenuated recombinant rIHNV-Gvhsv GFP virus with macrophages from rainbow trout (*Oncorhynchus mykiss*). *Veterinary Immunology and Immunopathology* 140, 119-129.

Salonius K., Siderakis C., MacKinnon A.M. and Griffiths S.G. (2005). Use of *Arthrobacter davidanieli* as a live vaccine against *Renibacterium salmoninarum* and *Piscirickettsia salmonis* in salmonids. *Developmental Biology* (Basel) 121, 189-197.

Salonius K., Simard N., Harland R., Ulmer J.B. (2007). The road to licensure of a DNA vaccine. *Current Opinion in Investigational Drugs* 8, 635-641.

Santander J., Mitra A. and Curtiss III R. (2011). Phenotype, virulence and immunogenicity of *Edwardsiella ictaluri* cyclic adenosine 3',5'-monophosphate receptor protein (Crp) mutants in catfish host. *Fish and Shellfish Immunology* 31, 1142-1153.

Sato H., Nakajima K., Maeno Y., Kamaishi T., Kamata T., Mori H., Kamei K., Takano R., Kudo K. and Hara S. (2000). Expression of YAV proteins and vaccination against viral ascites among cultured juvenile yellowtail. *Bioscience, Biotechnology, and Biochemistry* 64, 1494-1499.

- Schild G.C. (2005). DNA vaccines – Regulatory perspectives. *Progress in Fish Vaccinology, Develop. Biol.*, (ed. P. J. Midtlyng) Basel. Kaeger, pp. 215-261.
- Seo J.Y., Kim K.H., Kim S.G., Oh M.J., Nam S.W., Kim Y.T. and Choi T.J. (2006). Protection of flounder against hirame rhabdovirus (HIRRV) with a DNA vaccine containing the glycoprotein gene. *Vaccine* 24, 1009-1015.
- Shimmoto H., Kawai K., Ikawa T. and Oshima S. (2010). Protection of red sea bream *Pagrus major* against red sea bream iridovirus infection by vaccination with a recombinant viral protein. *Microbiology and Immunology* 54, 135-142.
- Shivappa R.B., McAllister P.E., Edwards G.H., Santi N., Evensen O. and Vakharia V.N. (2005). Development of a subunit vaccine for infectious pancreatic necrosis virus using a baculovirus insect/larvae system. *Developmental Biology* (Basel) 121, 165-174.
- Simon B., Nomellini J., Chiou P., Bingle W., Thornton J., Smit J. and Leong J.A. (2001). Recombinant vaccines against infectious hematopoietic necrosis virus: production by the *Caulobacter crescentus* S-layer protein secretion system and evaluation in laboratory trials. *Diseases of Aquatic Organisms* 44, 17-27.
- Sommerset I., Lorenzen E., Lorenzen N., Bleie H. and Nerland A.H. (2003). A DNA vaccine directed against a rainbow trout rhabdovirus induces early protection against a nodavirus challenge in turbot. *Vaccine* 21, 4661-4667.
- Sommerset I., Skern R., Biering E., Bleie H., Fiksdal I.U., Grove S. and Nerland A.H. (2005). Protection against Atlantic halibut nodavirus in turbot is induced by recombinant capsid protein vaccination but not following DNA vaccination. *Fish and Shellfish Immunology* 18, 13-29.
- Sun Y., Hu Y.H., Liu C.S. and Sun L. (2010). Construction and analysis of an experimental *Streptococcus iniae* DNA vaccine. *Vaccine* 28, 3905-3912.
- Sun Y., Liu C.S. and Sun L. (2010). Identification of an *Edwardsiella tarda* surface antigen and analysis of its immunoprotective potential as a purified recombinant subunit vaccine and a surface-anchored subunit vaccine expressed by a fish commensal strain. *Vaccine* 28, 6603-6608.
- Sun Y., Liu C.S. and Sun L. (2011a). Comparative study of the immune effect of an *Edwardsiella tarda* antigen in two forms: subunit vaccine vs DNA vaccine. *Vaccine* 29, 2051-2057.
- Sun Y., Liu C.S. and Sun L. (2011b). Construction and analysis of the immune effect of an *Edwardsiella tarda* DNA vaccine encoding a D15-like surface antigen. *Fish and Shellfish Immunology* 30, 273-279.
- Sun Y., Zhang M., Liu C.S., Qiu R. and Sun L. (2012). A divalent DNA vaccine based on Sia10 and OmpU induces cross protection against *Streptococcus iniae* and *Vibrio anguillarum* in Japanese flounder. *Fish and Shellfish Immunology* 32, 1216-1222.
- Takano T., Iwahori A., Hirono I. and Aoki T. (2004). Development of a DNA vaccine against hirame rhabdovirus and analysis of the expression of immune-related genes after vaccination. *Fish and Shellfish Immunology* 17(4), 367-374.
- Tan C.-W., Jesudhasan P.R.R. and Woo P.T.K. (2008). Towards a metalloprotease-DNA vaccine against piscine cryptobiosis caused by *Cryptobia salmositica*. *Parasitology Research* 102, 265-275.
- Temprano A., Riaño J., Yugueros J., González P., de Castro L., Villena A., Luengo J.M. and Naharro G. (2005). Potential use of a *Yersinia ruckeri* O1 auxotrophic *aroA* mutant as a live attenuated vaccine. *Journal of Fish Diseases* 28, 419-427.
- Thoulouze M.I., Bouguyon E., Carpentier C. and Brémont M. (2004). Essential role of the NV protein of *Novirhabdovirus* for pathogenicity in rainbow trout. *Journal of Virology* 78, 4098-4107.
- Thune R.L., Fernandez D.H., Hawke J.P. and Miller R. (2003). Construction of a safe, stable, efficacious vaccine against *Photobacterium damsela* ssp. *piscicida*. *Diseases of Aquatic Organisms* 57, 51-58.
- Tian J., Sun X., Chen X., Yu J., Qu L. and Wang L. (2008b). The formulation and immunisation of oral poly(DL-lactide-co-glycolide) microcapsules containing a plasmid vaccine against lymphocystis disease virus in Japanese flounder (*Paralichthys olivaceus*). *International Immunopharmacology* 8, 900-908.

Tian J., Yu J. and Sun X. (2008c). Chitosan microspheres as candidate plasmid vaccine carrier for oral immunisation of Japanese flounder (*Paralichthys olivaceus*). *Veterinary Immunology and Immunopathology* 126, 220-229.

Tian J. and Yu J. (2011). Poly(lactic-co-glycolic acid) nanoparticles as candidate DNA vaccine carrier for oral immunization of Japanese flounder (*Paralichthys olivaceus*) against lymphocystis disease virus. *Fish and Shellfish Immunology* 30, 109-117.

Tonheim T.C., Bøgwald J. and Dalmo R.A. (2008). What happens to the DNA vaccine in fish? A review of current knowledge. *Fish and Shellfish Immunology* 25, 1-18.

Traxler G.S., Anderson E., LaPatra S.E., Richard J., Shewmaker B. and Kurath G. (1999). Naked DNA vaccination of Atlantic salmon *Salmo salar* against IHNV. *Diseases of Aquatic Organisms* 38, 183-190.

Vaughan L.M., Smith P.R. and Foster T.J. (1993). An aromatic-dependent mutant of the fish pathogen *Aeromonas salmonicida* is attenuated in fish and is effective as a live vaccine against the salmonid disease furunculosis. *Infection and Immunity* 61, 2172-2181.

Vazquez-Juarez R.C., Gomez-Chiarri M., Barrera-Saldaña H., Hernandez-Saavedra N., Dumas S. and Ascencio F. (2005). Evaluation of DNA vaccination of spotted sand bass (*Paralabrax maculatofasciatus*) with two major outer-membrane protein-encoding genes from *Aeromonas veronii*. *Fish and Shellfish Immunology* 19, 153-163.

Verjan N., Ooi E.L., Nochi T., Kondo H., Hirono I., Aoki T., Kiyono H. and Yuki Y. (2008). A soluble nonglycosylated recombinant infectious hematopoietic necrosis virus (IHNV) G-protein induces IFNs in rainbow trout (*Oncorhynchus mykiss*). *Fish and Shellfish Immunology* 25, 170-180.

Vivas J., Riaño J., Carracedo B., Razquin B.E., López-Fierro P., Naharro G. and Villena A.J. (2004). The auxotrophic *aroA* mutant of *Aeromonas hydrophila* as a live attenuated vaccine against *A. salmonicida* infections in rainbow trout (*Oncorhynchus mykiss*). *Fish and Shellfish Immunology* 16, 193-206.

Vivas J., Razquin B., López-Fierro P. and Villena A.J. (2005). Modulation of the immune response to an *Aeromonas hydrophila* *aroA* live vaccine in rainbow trout: effect of culture media on the humoral immune response and complement consumption. *Fish and Shellfish Immunology* 18, 223-233.

Wang Q., Chen J., Liu R. and Jia J. (2011). Identification and evaluation of an outer membrane protein OmpU from a pathogenic *Vibrio harveyi* isolate as vaccine candidate in turbot (*Scophthalmus maximus*). *Letters in Applied Microbiology* 53, 22-29.

Wang H.R., Hu Y.H., Zhang W.W. and Sun L. (2009). Construction of an attenuated *Pseudomonas fluorescens* strain and evaluation of its potential as a cross-protective vaccine. *Vaccine* 27, 4047-4055.

Wilhelm V., Miquel A., Burzio L.O., Roseblatt M., Engel E., Valenzuela S., Parada G. and Valenzuela P.D. (2006). A vaccine against the salmonid pathogen *Piscirickettsia salmonis* based on recombinant proteins. *Vaccine* 24, 5083-5091.

Winton J.R. (1997). Immunization with viral antigens: infectious haematopoietic necrosis. In "Fish Vaccinology" (ed. by Gudding R., Lillehaug A., Midtlyng P.J. and Brown F.). *Developments in biological standardization*. Basel, Switzerland, Karger, vol. 90, p211-220.

Woolard S.N. and Kumaraguru U. (2010). Viral vaccines and CTL response. *Journal of Biomedicine and Biotechnology* 2010, 141657.

Xu L., Mourich D.V., Engelking H.M., Ristow S., Arnzen J. and Leong J.C. (1991). Epitope mapping and characterization of the infectious hematopoietic necrosis virus glycoprotein, using fusion proteins synthesized in *Escherichia coli*. *Journal of Virology* 65, 1611-1615.

Yang H., Chen J., Yang G., Zhang X.H., Liu R. and Xue X. (2009). Protection of Japanese flounder (*Paralichthys olivaceus*) against *Vibrio anguillarum* with a DNA vaccine containing the mutated zinc-metalloprotease gene. *Vaccine* 27, 2150-2155.

Yao K. and Vakharia V.N. (1998) Generation of infectious pancreatic necrosis virus from cloned cDNA. *Journal of Virology* 72, 8913-8920.

Yasuike M., Kondo H., Hirono I. and Aoki T. (2007). Difference in Japanese flounder, *Paralichthys olivaceus* gene expression profile following hirame rhabdovirus (HIRRV) G and N protein DNA

vaccination. *Fish and Shellfish Immunology* 23(3), 531-541.

Yasuike M., Kondo H., Hirono I. and Aoki T. (2011a). Gene expression profile of HIRRV G and N protein gene vaccinated Japanese flounder, *Paralichthys olivaceus* during HIRRV infection. *Comparative Immunology, Microbiology and Infectious Diseases* 34(2), 103-110.

Yeh H.Y. and Klesius P.H. (2011). Over-expression, purification and immune responses to *Aeromonas hydrophila* AL09-73 flagellar proteins. *Fish and Shellfish Immunology* 31, 1278-1283.

Zhang W.W., Sun K., Cheng S. and Sun L. (2008). Characterization of DegQVh, a serine protease and a protective immunogen from a pathogenic *Vibrio harveyi* strain. *Applied and Environmental Microbiology* 74, 6254-6262.

Section: 1.5. Fish Immunostilumants

Sakai M. (1999). Current research status of fish immunostimulants. *Aquaculture* 172, 63-92.

Kitao T. and Yoshida T. (1986). Effect of an immunopotentiator on *Aeromonas salmonicida* infection in rainbow trout (*Salmo gairdneri*). *Veterinary Immunology and Immunopathology* 12, 287-291.

Matsuo K. and Miyazano I. (1993). The influence of long-term administration of peptidoglycan on disease resistance and growth of juvenile rainbow trout. *Nippon Suisan Gakkaishi*, 59, 1377-1379.

Kajita Y., Sakai M., Atsuta S. and Kobayashi M. (1990). The immunomodulatory effects of levamisole on rainbow trout, *Oncorhynchus mykiss*. *Fish Pathology* 25, 93-98.

Robertsen B., Ehgstad R.E. and Jørgensen J.B. (1994). β -glucan as immunostimulants in fish. In "Modulators of Fish Immune Responses I" (ed by J.S. Stolen, T.C. Fletcher), 83-99 pp. SOS Publications, Fair Haven, NJ, USA.

Thompson K.D., Cachos A. and Inglis V. (1995). Immunomodulating effects of glucans and oxytetracycline in rainbow trout, *Oncorhynchus mykiss*, on serum lysozyme and protection. In "Diseases in Asian Aquaculture II" (ed by M. Shariff, R.P. Subasighe, J.R. Arthur), 433-439. pp. Fish Health Section, Asian Fisheries Society, Manila, Philippines.

Rørstad G., Aasjord P.M. and Robertsen B. (1993). Adjuvant effect of a yeast glucan in vaccines against furunculosis in Atlantic salmon (*Salmo salar* L.). *Fish and Shellfish Immunology* 3, 179-190.

Baulny M.O.D., Quentel C., Fournier V., Lamour F. and Gouvello R.L. (1996). Effect of long-term oral administration of β -glucan as an immunostimulant or an adjuvant on some non-specific parameters of the immune response of turbot *Scophthalmus maximus*. *Diseases of Aquatic Organism* 26, 139-147.

Aakre R., Wergeland H.I., Aasjord P.M. and Endersen C., (1994). Enhanced antibody response in Atlantic salmon (*Salmo salar* L.) to *Aeromonas salmonicida* cell wall antigens using a bacterin containing β -1, 3-M-glucan as adjuvant. *Fish and Shellfish Immunology* 4, 47-61.

Section: 2.1. Diagnosis -Antiserum Detection

Cunningham C. (Ed.) *Molecular Diagnosis of Salmonid Diseases*. Springer, 2002, XV, 347 p.

Adams A. and Thompson K.D. (2006). Biotechnology offers revolution to fish health management. *Trends in Biotechnology* 24(5), 201-205.

Tizard I.R. (2010). *Veterinary Immunology*. Elsevier Limited, Oxford.

Ellis A.E. (1988). (ed.) *Fish vaccination*. Academic Press., London.

Nho S.W., Shin G.W., Park S.B., Jang H.B., Cha I.S., Ha M.A., Kim Y.R., Park Y.K., Dalvi R.S., Kang B.J., Joh S.J. and Jung T.S. (2009). Phenotypic characteristics of *Streptococcus iniae* and *Streptococcus parauberis* isolated from olive flounder (*Paralichthys olivaceus*). *FEMS Microbiology Letters* 293(1), 20-27.

Lipman N.S., Jackson L.R., Trudel L.J. and Weis-Garcia F. (2005). Monoclonal versus polyclonal antibodies: distinguishing characteristics, applications, and information resources. *ILAR Journal* 46(3), 258-268.

Roberson B.S. (1993). Bacterial agglutination. PP. 81-86. In *Techniques in Fish immunology FITC-1*.

Joannes S., Thelma F., Douglas A., Stephen K. and Andrew R. (eds.), SOS publication.

Marja E. Koivunen and Richard L. (2006). Krogsrud Principles of Immunochemical Techniques Used in Clinical Laboratories. *Laboratory Medicine* 37, 490-497.

Anderson D.P. (1993). Fluorescent Antibody Test. PP 1-8. In *Techniques in Fish immunology FITC-1*. Joannes S., Thelma F., Douglas A., Stephen K. and Andrew R. (eds.), SOS publication.

Thuvander A., Johannisson A. and Grawe J. (1992). Flow Cytometry in Fish Immunology. PP 19-26. *Techniques in Fish immunology FITC-2*. Joannes S., Thelma F., Douglas A., Stephen K. and Andrew R. (eds.), SOS publication.

Kim M.S., Kim D.S. and Kim K.H. (2011). Oral immunization of olive flounder (*Paralichthys olivaceus*) with recombinant live viral hemorrhagic septicemia virus (VHSV) induces protection against VHSV infection. *Fish and Shellfish Immunology* 31(2), 212-216.

Alexandra A. (2006). Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) to detect and quantify Bacterial Pathogens in Fish Tissues. In: *Techniques in Fish immunology FITC-2*, pp.177-185, Joannes S., Thelma F., Douglas A., Stephen K. and Andrew R. (eds.), SOS publication.

Oh J.S., Ha G.W., Cho Y.S., Kim M.J., An D.J., Hwang K.K., Lim Y.K., Park B.K., Kang B. and Song D.S. (2006). One-step immunochromatography assay kit for detecting antibodies to canine parvovirus. *Clinical and Vaccine Immunology* 13, 520-524.

Adams A. and Thompson K.D. (2011). Development of diagnostics for aquaculture: challenges and opportunities. *Aquaculture Research* 42, 93-102.

Section: 2.2. Diagnosis – PCR Detection

Aoki T. and Hirono I. (1995). Detection of the fish-pathogenic bacteria *Edwardsiella tarda* by polymerase chain reaction. Proceedings of the international symposium on biotechnology applications in aquaculture 10, 135-146.

Aoki T., Hirono I. and Hayashi A. (1995). The fish-pathogenic bacterium *Pasteurella piscicida* detected by the polymerase chain reaction (PCR). In *Disease in Asian Aquaculture II*. Shariff M., Arthur J.R. and Subasinghe R.P. (eds.), PP. 347-353. Fish health section, Asian fisheries society, Manila.

Aoki T., Ikeda D., Katagiri T. and Hirono I. (1997). Rapid detection of the fish-pathogenic bacterium *Pasteurella piscicida* by polymerase chain reaction targeting nucleotide sequences of the species-specific plasmid pZP1. *Fish Pathology* 32, 143-151.

Aoki T., Park C.-I., Yamashita H. and Hirono I. (2000). Species-specific polymerase chain reaction primers for *Lactococcus garvieae*. *Journal of Fish Diseases* 23, 1-6.

Arakawa C.K., Deering, R.E., Higman, K.H., Oshima, K.H., Ohara, P.J. and Winton, J.R. (1990). Polymerase chain reaction (PCR) amplification of a nucleoprotein gene sequence of infectious haematopoietic necrosis virus. *Diseases of Aquatic Organisms* 8, 165-170.

Argenton F., De Mas S., Malocco C., Dalla Valle L., Giorgetti G. and Colombo L. (1996). Use of random DNA amplification to generate specific molecular probes for hybridization tests and PCR-based diagnosis of *Yersinia ruckeri*. *Diseases of Aquatic Organisms* 24, 121-127.

Arias C.R., Garay E. and Aznar R. (1995). Nested PCR method for rapid and sensitive detection of *Vibrio vulnificus* in fish, sediments, and water. *Applied and Environmental Microbiology* 61, 3476-3478.

Aso Y., Wani J., Antonio S.-K.D. and Yoshimizu M. (2001). Detection and identification of *Oncorhynchus masou* virus (OMV) disease by polymerase chain reaction (PCR). *Bulletin of Fisheries Sciences Hokkaido University* 52, 111-116.

Avendaño-Herrera R., Magariños B., Toranzo A.E., Beaz R. and Romalde J.L. (2004). Species-specific polymerase chain reaction primer sets for the diagnosis of *Tenacibaculum maritimum* infection. *Diseases of Aquatic Organisms* 62, 75-83.

Bader J.A. and Shotts E.B. (1998). Identification of *Flavobacterium* and *Flexibacter* species by species-specific polymerase chain reaction primers to the 16S ribosomal RNA gene. *Journal of Aquatic Animal Health* 10, 311-319.

Bader J.A., Shoemaker C.A. and Klesius P.H. (2003). Rapid detection of columnaris disease in channel catfish (*Ictalurus punctatus*) with a new species-specific 16-S rRNA gene-based PCR primer for *Flavobacterium columnare*. *Journal of Microbiological Methods* 52, 209-220.

Bercovier H., Fishman Y., Nahary R., Sinai S., Zlotkin A., Eynogor M., Gilad O., Eldar A. and Hedrick R.P. (2005). Cloning of the koi herpesvirus (KHV) gene encoding thymidine kinase and its use for a highly sensitive PCR based diagnosis. *BMC Microbiology* 5:13.

Berridge B.R., Fuller J.D., de Azavedo J., Low D.E., Bercovier H. and Frelter P.F. (1998). Development of specific nested oligonucleotide PCR primers for the *Streptococcus iniae* 16S-23S ribosomal DNA intergenic spacer. *Journal of Clinical Microbiology* 36, 2778-2781.

Blanco M.M., Gibello A., Vela A.I., Moreno M.A., Domínguez L. and Fernandez-Garayzábal J.F. (2002). PCR detection and PFGE DNA macrorestriction analyses of clinical isolates of *Pseudomonas anguilliseptica* from winter disease outbreaks in sea bream *Sparus aurata*. *Diseases of Aquatic Organisms* 50, 19-27.

Bowers R.M., Lapatra S.E. and Dhar A.K. (2008). Detection and quantitation of infectious pancreatic necrosis virus by real-time reverse transcriptase-polymerase chain reaction using lethal and non-lethal tissue sampling. *Journal of Virological Methods* 147, 226-234.

Brown L.L., Iwama G.K., Evelyn T.P.T., Nelson W.S. and Levine R.P. (1994). Use of the polymerase chain reaction (PCR) to detect DNA from *Renibacterium salmoninarum* within individual salmonid eggs. *Diseases of Aquatic Organisms* 18, 165-171.

Bruchhof B., Marquardt O. and Enzmann P.J. (1995). Differential diagnosis of fish pathogenic rhabdoviruses by reverse transcriptase-dependent polymerase chain reaction. *Journal of Virological Methods* 55, 111-119.

Cano I., Ferro P., Alonso M.C., Bergmann S.M., Römer-Oberdörfer A., Garcia-Rosado E., Castro D. and Borrego J.J. (2007). Development of molecular techniques for detection of lymphocystis disease virus in different marine fish species. *Journal of Applied Microbiology* 102, 32-40.

Cepeda C., García-Márquez S. and Santos Y. (2003). Detection of *Flexibacter maritimus* in fish tissue using nested PCR amplification. *Journal of Fish Diseases* 26, 65-70.

Chico V., Gomez N., Estepa A. and Perez L. (2006). Rapid detection and quantitation of viral hemorrhagic septicemia virus in experimentally challenged rainbow trout by real-time RT-PCR. *Journal of Virological Methods* 132, 154-159.

Coleman S.S., Melanson D.M., Biosca E.G. and Oliver J.D. (1996). Detection of *Vibrio vulnificus* biotypes 1 and 2 in eels and oysters by PCR amplification. *Applied and Environmental Microbiology* 62, 1378-1382.

Crisafi F., Denaro R., Genovese M., Cappello S., Mancuso M. and Genovese L. (2011). Comparison of 16SrDNA and *toxR* genes as targets for detection of *Vibrio anguillarum* in *Dicentrarchus labrax* kidney and liver. *Research in Microbiology* 162, 223-230.

Dalla Valle L., Zanella L., Patarnello P., Paolucci L., Belvedere P. and Colombo L. (2000). Development of a sensitive diagnostic assay for fish nervous necrosis virus based on RT-PCR plus nested PCR. *Journal of Fish Diseases* 23, 321-328.

Darwish A.M., Ismaiel A.A., Newton J.C. and Tang J. (2004). Identification of *Flavobacterium columnare* by a species-specific polymerase chain reaction and renaming of ATCC43622 strain to *Flavobacterium johnsoniae*. *Molecular and Cellular Probes* 18, 421-427.

Devold M., Krossøy B., Aspehaug V. and Nylund A. (2000). Use of RT-PCR for diagnosis of infectious salmon anaemia virus (ISAV) in carrier sea trout *Salmo trutta* after experimental infection. *Diseases of Aquatic Organisms* 40, 9-18.

Forsman P., Tilsala-Timisjärvi A. and Alatossava T. (1997). Identification of staphylococcal and streptococcal causes of bovine mastitis using 16S-23S rRNA spacer regions. *Microbiology* 143, 3491-3500.

Gibello A., Blanco M.M., Moreno M.A., Cutuli M.T., Domenech A., Domínguez L. and Fernández-Garayzábal J.F. (1999). Development of a PCR assay for detection of *Yersinia ruckeri* in tissues of inoculated and naturally infected trout. *Applied and Environmental Microbiology* 65, 346-350.

- Gilad O., Yun S., Andree K.B., Adkison M.A., Zlotkin A., Bercovier H., Eldar A. and Hedrick R.P. (2002). Initial characteristics of koi herpesvirus and development of a polymerase chain reaction assay to detect the virus in koi, *Cyprinus carpio koi*. *Diseases of Aquatic Organisms* 48, 101-108.
- Gray W.L., Williams R.J., Jordan, R.L. and Griffin B.R. (1999). Detection of channel catfish virus DNA in latently infected catfish. *Journal of General Virology* 80, 1817-1822.
- Gray W.L., Williams R.J., Jordan R.L. and Griffin B.R. (1999). Detection of channel catfish virus DNA in latently infected catfish. *Journal of General Virology* 80, 1817-1822.
- Grayson T.H., Cooper L.F., Atienzar F.A., Knowles M.R. and Gilpin M.L. (1999) Molecular differentiation of *Renibacterium salmoninarum* isolates from worldwide locations. *Applied and Environmental Microbiology* 65, 961-968.
- Gustafson C.E., Thomas C.J. and Trust T.J. (1992). Detection of *Aeromonas salmonicida* from fish by using polymerase chain reaction amplification of the virulence surface array protein gene. *Applied and Environmental Microbiology* 58, 3816-3825.
- Gustafson C.E., Alm R.A. and Trust T.J. (1993). Effect of heat denaturation of target DNA on the PCR amplification. *Gene* 123, 241-244.
- Hassan A.A., Khan I.U. and Lammler C. (2003). Identification of *Streptococcus dysgalactiae* strains of Lancefield's group C, G and L by polymerase chain reaction. *Journal of Veterinary Medicine B* 50, 161-165.
- Hill W.E., Keasler S.P., Trucksess M.W., Feng P., Kaysner C.A. and Lampel K.A. (1991). Polymerase chain reaction identification of *Vibrio vulnificus* in artificially contaminated oysters. *Applied and Environmental Microbiology* 57, 707-711.
- Hirono I., Masuda T. and Aoki T. (1996). Cloning and detection of the hemolysin gene of *Vibrio anguillarum*. *Microbial Pathogenesis* 21, 173-182.
- Hodneland K. and Endresen C. (2006). Sensitive and specific detection of Salmonid alphavirus using real-time PCR (TaqMan). *Journal of Virological Methods* 131, 184-192.
- Holopainen R., Honkanen J., Jensen B.B., Ariel E. and Tapiovaara H. (2011). Quantitation of ranaviruses in cell culture and tissue samples. *Journal of Virological Methods* 171, 225-233.
- Hussein M.M.A. and Hatai K. (2006). Multiplex PCR for detection of *Lactococcus garvieae*, *Streptococcus iniae* and *S. dysgalactiae* in cultured yellowtail. *Aquatic Sciences* 54, 269-274.
- Iwamoto Y., Suzuki Y., Kurita A., Watanabe Y., Shimizu T., Ohgami H. and Yanagihara Y. (1995). Rapid and sensitive PCR detection of *Vibrio trachuri* pathogenic to Japanese horse mackerel (*Trachurus japonicus*). *Microbiology and Immunology* 39, 1003-1006.
- Keeling S.E., Johnston C., Wallis R., Brosnahan C.L., Gudkovs N. and McDonald W.L. (2012). Development and validation of real-time PCR for the detection of *Yersinia ruckeri*. *Journal of Fish Diseases* 35, 119-125.
- Kim D.G., Bae J.Y., Hong G.E., Min M.K., Kim J.K. and Kong I.S. (2008). Application of the *rpoS* gene for the detection of *Vibrio anguillarum* in flounder and prawn by polymerase chain reaction. *Journal of Fish Diseases* 31, 639-647.
- Kitamura S., Jung S.J. and Oh M.J. (2006). Differentiation of lymphocystis disease virus genotype by multiplex PCR. *Journal of Microbiology* 44, 248-253.
- Kox L.F., van Leeuwen J., Knijper S., Jansen H.M. and Kolk A.H. (1995). PCR assay based on DNA coding for 16S rRNA for detection and identification of mycobacteria in clinical samples. *Journal of Clinical Microbiology* 33, 3225-3233.
- Koutná M., Veselý T., Psikal I. and Hůlová J. (2003). Identification of spring viraemia of carp virus (SVCV) by combined RT-PCR and nested PCR. *Diseases of Aquatic Organisms* 55, 229-235.
- Kox L.F., Jansen H.M., Kuijper S. and Kolk A.H. (1997). Multiplex PCR assay for immediate identification of the infecting species in patients with mycobacterial disease. *Journal of Clinical Microbiology* 35, 1492-1498.

- Kurita J., Nakajima K., Hirono I. and Aoki T. (1998). Polymerase chain reaction (PCR) amplification of DNA of red sea bream iridovirus (RSIV). *Fish Pathology* 33, 17–23.
- Liu Z., Teng Y., Liu H., Jiang Y., Xie X., Li H., Lv J., Gao L., He J., Shi X., Tian F., Yang J. and Xie C. (2008). Simultaneous detection of three fish rhabdoviruses using multiplex real-time quantitative RT-PCR assay. *Journal of Virological Methods* 149, 103-109.
- López-Vázquez C., Dopazo C.P., Oliveira J.G., Barja J.L. and Bandín I. (2006). Development of a rapid, sensitive and non-lethal diagnostic assay for the detection of viral haemorrhagic septicaemia virus. *Journal of Virological Methods* 133, 167-174.
- Magnússon H.B., Fridjónsson O.H., Andrésson O.S., Benediksdóttir E., Guðmundsdóttir S. and Andrésdóttir V. (1994). *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease in salmonid fish, detected by nested reverse transcription-PCR of 16S rRNA sequences. *Applied and Environmental Microbiology* 60, 4580-4583.
- Mata A.I., Blanco M.M., Domínguez L., Fernández-Garayzábal J.F. and Gibello A. (2004a). Development of a PCR assay for *Streptococcus iniae* based on the lactate oxidase (*lctO*) gene with potential diagnostic value. *Veterinary Microbiology* 101, 109-116.
- Mata A.I., Gibello A., Casamayor A., Blanco M.M., Domínguez L. and Fernández-Garayzábal J.F. (2004b). Multiplex PCR assay for detection of bacterial pathogens associated with warm-water Streptococcosis in fish. *Applied and Environmental Microbiology* 70, 3183-3187.
- Matsuoka, M., Hirose K, Soumyo Y., Nishizawa T. Muroga K. (1997). Detection of *Photobacterium damsela* from Yellowtail by RT-PCR. *Journal of the Faculty of Applied Biological Science, Hiroshima University*, 36, 139-146. (in Japanese)
- McIntosh D., Meaden P.G. and Austin B. (1996). A simplified PCR-based method for the detection of *Renibacterium salmoninarum* utilizing preparations of rainbow trout (*Oncorhynchus mykiss*, Walbaum) lymphocytes. *Applied and Environmental Microbiology* 62, 3929-3932.
- Miller T.A., Rapp J., Wasthuber U., Hoffmann R.W. and Enzmann P.J. (1998). Rapid and sensitive reverse transcriptase-polymerase chain reaction based detection and differential diagnosis of fish pathogenic rhabdoviruses in organ samples and cultured cells. *Diseases of Aquatic Organisms* 34, 13-20.
- Miriam A., Griffiths S.G., Lovely J.E. and Lynch W.H. (1997). PCR and probe-PCR assays to monitor broodstock Atlantic salmon (*Salmo salar* L.) ovarian fluid and kidney tissue for presence of DNA of the fish pathogen *Renibacterium salmoninarum*. *Journal of Clinical Microbiology* 35, 1322-1326.
- Mitsui S., Iida T., Yoshida T., Hirono I. and Aoki T. (2004). PCR-based detection of the causative agent of bacterial hemolytic jaundice in yellowtail. *Fish Pathology*, 39, 43-45. (in Japanese)
- Miyata M., Inglis V. and Aoki T. (1996). Rapid identification of *Aeromonas salmonicida* subspecies *salmonicida* by the polymerase chain reaction. *Aquaculture* 141, 13-24.
- Miyoshi Y. and Suzuki S. (2003). A PCR method to detect *Nocardia seriolae* in fish samples. *Fish Pathology* 38, 93-97.
- O'Brien D., Mooney J., Ryan D., Powell E., Hiney M., Smith P.R. and Powell R. (1994). Detection of *Aeromonas salmonicida*, causal agent of furunculosis in salmonid fish, from the tank effluent of hatchery-reared Atlantic salmon smolts. *Applied and Environmental Microbiology* 60, 3874-3877.
- Orpetveit I., Mikalsen A.B., Sindre H., Evensen O., Dannevig B.H. and Midtlyng P.J. (2010). Detection of infectious pancreatic necrosis virus in subclinically infected Atlantic salmon by virus isolation in cell culture or real-time reverse transcription polymerase chain reaction: influence of sample preservation and storage. *Journal of Veterinary Diagnostic Investigation* 22, 886-895. Erratum in: *Journal of Veterinary Diagnostic Investigation* 23, 395.
- Oshima S., Hata J., Hirasawa N., Ohtaka T., Hirono I., Aoki T. and Yamashita S. (1998). Rapid diagnosis of red sea bream iridovirus infection using the polymerase chain reaction. *Diseases of Aquatic Organisms* 32, 87-90.
- Oshima S., Hata J., 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, 15-19.

Palmer L.J., Hogan N.S. and 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.

Puttinaowarat S., Thompson K.D., Kolk A. and Adams A. (2002) Identification of *Mycobacterium* spp. Isolated from snakehead, *Channa striata* (Fowler), and Siamese fighting fish, *Betta splendens* (Regan), using polymerase chain reaction-reverse cross blot hybridization (PCR-RCBH). *Journal of Fish Diseases* 25, 235-243.

Roach J.C., Levett P.N. and Lavoie M.C. (2006). Identification of *Streptococcus iniae* by commercial bacterial identification systems. *Journal of Microbiological Methods* 67, 20-26.

Rodríguez S., Alonso M. and Pérez-Prieto S.I. (2001). Detection of infectious pancreatic necrosis virus (IPNV) from leukocytes of carrier rainbow trout (*Oncorhynchus mykiss*). *Fish Pathology* 36, 139-146.

Sun Y., Yue Z., Liu H., Zhao Y., Liang C., Li Y., Shi X., Wu B., Xu B., Deng M., Zhu L. and Wang Z. (2010). Development and evaluation of a sensitive and quantitative assay for hirame rhabdovirus based on quantitative RT-PCR. *Journal of Virological Methods* 169, 391-396.

Toyama T., Kita-Tsukamoto K. and Wakabayashi H. (1994). Identification of *Cytophaga psychrophila* by PCR targeted 16S ribosomal RNA. *Fish Pathology* 29, 271-275.

Toyama T., Kita-Tsukamoto K. and Wakabayashi H. (1996). Identification of *Flexibacter maritimus*, *Flavobacterium branchiophilum* and *Cytophaga columnaris* by PCR targeted 16S ribosomal DNA. *Fish Pathology* 31, 25-31.

Urdaci M.C., Chakroun C., Faure D. and Bernardet J.F. (1998). Development of a polymerase chain reaction assay for identification and detection of the fish pathogen *Flavobacterium psychrophilum*. *Research in Microbiology* 149, 519-30.

Welker T.L., Shoemaker C.A., Arias C.R. and Klesius P.H. (2005). Transmission and detection of *Flavobacterium columnare* in channel catfish *Ictalurus punctatus*. *Diseases of Aquatic Organisms* 63, 129-138.

Williams K., Blake S., Sweeney A., Singer J.T. and Nicholson B.L. (1999). Multiplex reverse transcriptase PCR assay for simultaneous detection of three fish viruses. *Journal of Clinical Microbiology* 37, 4139-4141.

Williams M.L. and Lawrence M.L. (2010). Verification of an *Edwardsiella ictaluri*-specific diagnostic PCR. *Letters in Applied Microbiology* 50, 153-157.

Xiao P., Mo Z.L., Mao Y.X., Wang C.L., Zou Y.X. and Li J. (2009). Detection of *Vibrio anguillarum* by PCR amplification of the *empA* gene. *Journal of Fish Diseases* 32, 293-296.

Yuasa K., Kurita J., Kawana M., Kiryu I., Oseko N. and Sano M. (2012). Development of mRNA-specific RT-PCR for the detection of koi herpesvirus (KHV) replication stage. *Diseases of Aquatic Organisms* 100, 11-18.

Zhang L., Luo Q., Fang Q. and Wang Y. (2010). An improved RT-PCR assay for rapid and sensitive detection of grass carp reovirus. *Journal of Virological Methods* 169, 28-33.

Zhou S.M., Fan Y., Zhu X.Q., Xie M.Q. and Li A.X. (2011). Rapid identification of *Streptococcus iniae* by specific PCR assay utilizing genetic markers in ITS rDNA. *Journal of Fish Diseases* 34, 265-271.

Zlotkin A., Eldar A., Ghittino C. and Bercovier H. (1998a). Identification of *Lactococcus garvieae* by PCR. *Journal of Clinical Microbiology* 36, 983-985.

Zlotkin A., Hershko H. and Eldar A. (1998b). Possible transmission of *Streptococcus iniae* from wild fish to cultured marine fish. *Applied and Environmental Microbiology* 64, 4065-4067.

Section: 2.3. Loop Mediated Isothermal Amplification (LAMP) Method

Notomi. T., Okayama. H., Masubuchi. H., Yonekawa. T., Watanabe. K., Amino. N., and Hase. T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research* 28, E63.

Mori. Y., Nagamine. K., Tomita. N. and Notomi. T. (2001). Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. *Biochemistry and*

Biophysics Research Communications 289, 150–154.

Savan. R., Igarashi. A., Matsuoka. S., and Sakai. M. (2004). Sensitive and rapid detection of edwardsiellosis in fish by a loop-mediated isothermal amplification method. *Applied and Environmental Microbiology* 70, 621-624.

Itano. T., Kawakami. H., Kono. T., and Sakai. M. (2006). Detection of fish nocardiosis by loop-mediated isothermal amplification. *Journal of Applied Microbiology* 100, 1381-1387.

Fall. J., Chakraborty. G., Kono. T., Maeda. M., Itami. T. and Sakai. M. (2008). Establishment of loop-mediated isothermal amplification method (LAMP) for detection of *Vibrio nigripulchritudo* in shrimp. *FEMS Microbiology Letters* 228, 171–177.

Gunimaladevi. I., Kono. T., Venugopal. M.N., and Sakai. M. (2004). Detection of koi herpesvirus in common carp, *Cyprinus carpio* L., by loop-mediated isothermal amplification. *Journal of Fish Diseases* 27, 583-589.

Kono. T., Savan. R., Sakai. M., and Itami. T. (2004). Detection of white spot syndrome virus in shrimp by loop-mediated isothermal amplification. *Journal of Virological Methods* 115, 59-65.

Yoshino. M., Watari. H., Kojima. T. and Ikedo. M. (2006). Sensitive and rapid detection of koi herpesvirus by LAMP method. *Fish Pathology* 41, 19-27.

Caipang. C.M., Haraguchi. I., Ohira. T., Hirono. I., and Aoki. T. (2004). Rapid detection of a fish iridovirus using loop-mediated isothermal amplification (LAMP). *Journal of Virological Methods* 121, 155-161.

Sun. Z-F., Hu. C-Q., Ren. C-H. and Shen. Q. (2006). Sensitive and rapid detection of infectious hypodermal and hematopoietic necrosis virus (IHHNV) in shrimps by loop-mediated isothermal amplification. *Journal of Virological Methods* 131, 41–46.

Gunimaladevi. I., Kono. T., Lapatra. S.E., and Sakai. M. (2005). A loop mediated isothermal amplification (LAMP) method for detection of infectious hematopoietic necrosis virus (IHHNV) in rainbow trout (*Oncorhynchus mykiss*). *Archive Virology* 150, 899-909.

Saliman. H., and El-Matbouli. M. (2006). Reverse transcription loop-mediated isothermal amplification (RT-LAMP) for rapid detection of viral hemorrhagic septicaemia virus (VHS). *Veterinary Microbiology* 114, 205-213.

Mekata. T., Kono. T., Savan. R., Sakai. M., Kasornchandra. J., Yoshida. T., and Itami. T. (2006). Detection of yellow head virus in shrimp by loop-mediated isothermal amplification (LAMP). *Journal of Virological Methods* 135, 151-156.

Shivappa. R.B., Savan. R., Kono. T., Sakai. M., Emmenegger. E., Kurath. G. and Levine. J.F. (2008). Detection of spring viraemia of carp virus (SVCV) by loop-mediated isothermal amplification (LAMP) in koi carp, *Cyprinus carpio* L. *Journal of Fish Diseases* 31, 249-258.

El-Matbouli. M., and Soliman. H. (2005a). Rapid diagnosis of *Tetracapsuloides bryosalmonae*, the causative agent of proliferative kidney disease (PKD) in salmonid fish by a novel DNA amplification method, loop-mediated isothermal amplification (LAMP). *Parasitological Research* 96, 277-284.

El-Matbouli. M., and Soliman. H. (2005b). Development of a rapid assay for the diagnosis of *Myxobolus cerebralis* in fish and oligochaetes using loop-mediated isothermal amplification. *Journal of Fish Diseases* 28, 549-557.

(Sudhakaran. R., Mekata. T., Kono. T., Supamattaya. K., Linh. N.T.H., Sakai. M., and Itami. T. (2008). Rapid detection and quantification of infectious hypodermal and hematopoietic necrosis virus (IHHNV) by real-time loop-mediated isothermal amplification. *Fish Pathology* 43, 170-173.

Mekata. T., Sudhakaran. R., Kono. T., Supamattaya. K., Lihn. NTH., Sakai. M. and Itami. T. (2009a). Real-time quantitative loop-mediated isothermal amplification as a simple method for detecting white spot syndrome virus. *Letters in Applied Microbiology* 48, 25–32.

Mekata. T., Sudhakaran. R., Kono. T., Utaynapun. K., Supamattaya. K., Suzuki. Y., Sakai. M., and Itami. T. (2009b). Real-time reverse transcriptase loop-mediated isothermal amplification method for rapid detection of yellow head virus in shrimp. *Journal of Virological Methods* 162, 81-87.

Section: 3.1. Development of Disease-Resistant Fish Using Marker-Assisted Selection

Coimbra M.R.M., Kobayashi K., Koretsugu S., Hasegawa O., Ohara E., Ozaki A., Sakamoto T., Naruse K., Okamoto N. (2003). A genetic linkage map of the Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, 220, 203-218.

Fuji K., K. Kobayashi, O. Hasegawa, M.R.M. Coimbra, T. Sakamoto and N. Okamoto (2006). Identification of a single major genetic locus controlling the resistance to lymphocystis disease in Japanese flounder (*Paralichthys olivaceus*). *Aquaculture*, 254, 203-210.

Fuji K., Hasegawa O., Honda K., Kumasaka K., Sakamoto T., Okamoto N. (2007). Marker-assisted breeding of a lymphocystis disease-resistant Japanese flounder (*Paralichthys olivaceus*). *Aquaculture*, 272, 291-295.

Gilbey J., Verspoor E., McLay A., Houlihan D. (2004). A microsatellite linkage map for Atlantic salmon (*Salmo salar*). *Animal Genetics*, 35, 98-105.

Houston R.D., Haley C.S., Hamilton A, Guy D.R., Tinch A.E., Taggart J.B., McAndrew B.J., Bishop S.C. (2008). Major quantitative trait loci affect resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*). *Genetics*, 178, 1109–1115.

Houston R.D., Davey J.W., Bishop S.C., Lowe N.R., Mota-Velasco J.C., Hamilton A., Guy D.R., Tinch A.E., Thomson M.L., Blaxter M.L, Gharbi K., Bron J.E., Taggart J.B. (2012). Characterisation of QTL-Linked and Genome-Wide Restriction Site-Associated DNA (RAD) markers in farmed Atlantic salmon. *BMC Genomics*, 13, 244.

Lien S, Gidskehaug L., Moen T., Hayes B.J., Berg P.R., Davidson W.S., Omholt S.W., M.P. Kent. (2011). A dense SNP-based linkage map for Atlantic salmon (*Salmo salar*) reveals extended chromosome homeologies and striking differences in sex-specific recombination patterns. *BMC genomics*, 12, 615.

Moen T, Hoyheim B., Munck H., Gomez-Raya L. (2004). A linkage map of Atlantic salmon (*Salmo salar*) reveals an uncommonly large difference in recombination rate between the sexes. *Animal genetics*, 35, 81-92.

Moen T, Hayes B., Baranski M., Berg P.R., Kjøglum S., Koop B.F., Davidson W.S., Omholt S.W. Lien S. (2008). A linkage map of the Atlantic salmon (*Salmo salar*) based on EST-derived SNP markers. *BMC genomics*, 9, 223.

Moen T., Baranski M., Sonesson A.K., Kjøglum S. (2009). Confirmation and fine-mapping of a major QTL for resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*): population-level associations between markers and trait. *BMC Genomics*, 10, 368.

Ozaki A., Yoshida K., Fuji K., Kubota S., Kai W., Aoki J., Kawabata Y., Suzuki J., Akita K., Koyama T., Nakagawa M., Hotta T., Tsuzaki T., Okamoto N., Araki K., Sakamoto T. (2013). Quantitative trait loci (QTL) associated with resistance to a monogenean parasite (*Benedenia seriolae*) in yellowtail (*Seriola quinqueradiata*) through genome wide analysis. *PLoS One*, 8 (6), e6498.

Sanchez C.C., Fuji K., Ozaki A., Hasegawa O., Sakamoto T., Morishima K., Nakayama I., Fujiwara A., Masaoka T., Okamoto H., Hayashida K., Tagami M., Kawai J., Hayashizaki Y., Okamoto N. (2010). A second generation genetic linkage map of Japanese flounder (*Paralichthys olivaceus*). *BMC Genomics*, 11, 554.

Section: 3.2. Establishment of Disease-Resistant Fish

Dunham R.A. (2009) Transgenic fish resistant to infectious diseases, their risk and prevention of escape into the environment and future candidate genes for disease transgene manipulation. *Comparative Immunology, Microbiology and Infectious Diseases* 32, 139-61.

Dunham R.A., Warr G.W., Nichols A., Duncan P.L., Argue B., and Middleton D. (2002) Enhanced bacterial disease resistance of transgenic channel catfish *Ictalurus punctatus* possessing cecropin genes. *Marine Biotechnology* 4, 338–344.

Sarmasik A., Warr G. and Chen T.T. (2002) Production of transgenic medaka with increased resistance to bacterial pathogens. *Marine Biotechnology* 4, 310–322.

Mao W., Wang Y., Wang W., Wu B., Feng J. and Zhu Z. (2004) Enhanced resistance to *Aeromonas hydrophila* infection and enhanced phagocytic activities in human lactoferrin-transgenic grass carp (*Ctenopharyngodon idellus*). *Aquaculture* 242, 93–103.

Yazawa R., Hirono I. and Aoki T. (2006) Transgenic zebrafish expressing chicken lysozyme show resistance against bacterial diseases. *Transgenic Research* 15, 385–391.

Su J., Yang C., Zhu Z., Wang Y., Jang S. and Liao L. (2009) Enhanced grass carp reovirus resistance of Mx-transgenic rare minnow (*Gobiocypris rarus*). *Fish and Shellfish Immunology* 26, 828-35.

Hsieh J.C., Pan C.Y. and Chen J.Y. (2010) Tilapia hepcidin (TH)2-3 as a transgene in transgenic fish enhances resistance to *Vibrio vulnificus* infection and causes variations in immune-related genes after infection by different bacterial species. *Fish and Shellfish Immunology* 29, 430-439.

Peng K.C., Pan C.Y., Chou H.N. and Chen J.Y. (2010) Using an improved Tol2 transposon system to produce transgenic zebrafish with epinecidin-1 which enhanced resistance to bacterial infection. *Fish and Shellfish Immunology* 28, 905–917.

Fletcher G.L., Hobbs R.S., Evans R.P., Shears M.A., Hahn A.L. and Hew C.L. (2011) Lysozyme transgenic Atlantic salmon (*Salmo salar* L.). *Aquaculture Research* 42, 427-440.

Pan C.Y., Peng K.C., Lin C.H. and Chen J.Y. (2011) Transgenic expression of tilapia hepcidin 1-5 and shrimp chelonianin in zebrafish and their resistance to bacterial pathogens. *Fish and Shellfish Immunology* 31, 275-285.

Anderson E.D., Mourich D.V. and Leong J.C. (1996) Genetic immunization of rainbow trout (*Oncorhynchus mykiss*) against infectious hematopoietic necrosis virus. *Molecular Marine Biology and Biotechnology* 5, 114–122.

Yamamoto Y., Kabeya N., Higuchi K., Yatabe T., Tsunemoto K., Yazawa R., Kawamura T., Takeuchi Y. and Yoshizaki G. (2011) Establishment of a stable transgenic strain in a pelagic egg spawning marine teleost, Nibe croaker *Nibea mitsukurii*. *Aquaculture* 313, 42-49.

Biographical Sketches

Dr. Mamoru Yoshimizu is currently an invited teacher in Faculty of Fisheries Sciences, Hokkaido University. He obtained Ph.D. degree from Hokkaido University. Dr. Yoshimizu has been researching on fish disease and prevention of pathogens for many years.

Dr. Hisae Kasai is currently an associate professor in Faculty of Fisheries Sciences, Hokkaido University. She obtained Ph.D. degree from Hokkaido University in 2005. Dr. Kasai has been working on fish disease and food safety for many years.

Dr. Takashi Aoki is currently a visiting professor in the Consolidated Research Institute for Advanced Science and Medical Care, Waseda University, and he is also an emeritus professor for Tokyo University of Marine Science and Technology (TUMST). He obtained Ph.D. degree from The University of Tokyo in 1973. Dr. Aoki has been working on fish disease, chemotherapy and immunology for many years.

Dr. Mitsuru Ototake is currently a Director of Aquatic animal Health Division, National Research Institute of Aquaculture, Fisheries Research Agency. Dr. Ototake has always been interested in the problem of fish diseases, especially fish immunology and vaccination. He has served as a senior editor in Editorial Board of “Fish Pathology”.

Dr. Masahiro Sakai is currently a professor in Faculty of Agriculture, University of Miyazaki. He obtained PhD degrees from The University of Tokyo. Dr. Sakai has been working on fish immunostimulants and fish cytokines for many years.

Dr. Tae Sung Jung is a professor and charge of Lab. of Aquatic Animal Diseases, College of Veterinary Medicine Gyeongsang National University, South Korea. He completed his Ph.D. degree from Institute of Aquaculture, University of Stirling, UK in 1999. Dr Jung has been working on fish diseases and immunology for many years.

Dr. Jun-ichi Hikima is currently an associate professor in Department of Biochemistry and Applied Biosciences, Faculty of Agriculture, University of Miyazaki in Miyazaki, Japan. He obtained Ph.D. degree from Tokyo University of Fisheries (Current name is TUMSAT) in 2000. Dr. Hikima's major research covers infectious disease and immunity in fish..

Dr. Takashi Sakamoto is currently an associate professor in TUMST. He obtained Ph.D. degree from Tokyo University of Fisheries (Current name is TUMST) in 1996. Dr. Sakamoto has been working on fish disease and fish molecular genetics and breeding for many years.

Dr. Akiyuki Ozaki is currently a researcher in Aquatic Breeding and Genetics Technologies Division, National Research Institute of Aquaculture, Fisheries Research Agency in Japan. He obtained Ph.D. degree from Tokyo University of Fisheries (Current name is TUMSAT) in 2001. Dr. Ozaki has been working on fish heredity and breeding for many years.

Dr. Nobuaki Okamoto is currently a professor and a president for TUMST. He obtained Ph.D. degree from Hokkaido University in 1986. Dr. Okamoto has been working on fish diseases and molecular breeding for disease resistance for many years.

Dr. Ryosuke Yazawa is presently an assistant professor in Department of Marine Biosciences, TUMST. *Education:* 2005 Ph.D. (Fisheries Science) in Tokyo Univ. of Fisheries. *Postdoctoral experience:* 2005-2007. Centre for Biomedical Research, Univ. of Victoria, Canada. 2007-2011. Tokyo University of Marine Science and Technology, Japan. *Main research interest:* To establish the effective aquaculture productions system using marine biotechnology from the viewpoints of pathophysiology and reproductive physiology. *Current ongoing research:* 1. Development of surrogate broodstock technology in fish by germ cell transplantation. One of the final objectives of this study is making surrogate mackerel recipients producing bluefin tuna gametes. 2. Analysis of the molecular basis of inter-specific deference for the disease resistance in genus *Somber*.