DETECTION OF *Vibrio parahaemolyticus* INFECTION-RESISTANT BIOMARKERS IN TIGER GROUPER *Epinephelus fuscoguttatus* (Forsskål) USING QUANTITATIVE REAL-TIME PCR METHOD

By

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Grouper species which includes *Epinephelus fuscoguttatus* also known as tiger grouper is one of the valuable species with great economic value in Southeast Asian countries. Various infectious diseases occur in grouper aquaculture and often create severe problems leading to economic losses. Causative agents of vibriosis in aquaculture include *Vibrio parahaemolyticus*. *Vibrio parahaemolyticus* is a major foodborne pathogen and classified as a Gram-negative halophilic bacterium which commonly inhabits estuarine and marine environments and causes worldwide health problems on consumption. Therapeutic options with antibiotics and vaccines have disadvantages such as resistance and contamination from overuse. For centuries farmers have been aware of specific phenotypic attributes in plants and animals that provided a production advantage of one species over another. The advances in technology have permitted researchers to examine these features at the genetic and molecular levels. Selective breeding is a viable option to improve health of fish in culture. Feasible and rapid methods for identification of relevant biomarkers in groupers are factors that aid in management of its produce. Real time qPCR-based methods are convenient, easy to screen for epidemiological, genetic studies and applicable to routine high-throughput detection of large numbers of samples and can be used for quantitative gene expression of biomarkers in grouper organs and tissues. The purpose of this study is to confirm usefulness of previously identified biomarkers in selection of fish with disease resistance. Herein the potential biomarkers, the alpha-2-Macroglobulin (*α2M*), Parvalbumin Beta-2 Subunit 1 (*PVALB*) and Nattetin genes were selected. Primers were designed using Primer-BLAST from the NCBI website and synthesized by a local company. β-actin (*ACTB*), acted as the reference gene. Experimental infections of grouper with *V. parahaemolyticus* were conducted to assess expression levels of these genes in infected fish compared to controls. Healthy juveniles' grouper fish infected with *V. parahaemolyticus* were monitored for skin lesions in tissues and samples collected after 2 weeks. *Vibrio parahaemolyticus* infected grouper displayed significantly increased expression of alpha-2-Macroglobulin (*α2M*), Parvalbumin Beta-2 Subunit 1 (*PVALB*), and Nattectin

in blood, liver and spleen compared to before infection. Infection susceptible fish was based on the development of skin lesion more than five millimeter (>5mm) in diameter while none was observed on infection resistant fish. When infection-resistant fish was compared to fish infection-susceptible for these three genes *Nattectin*, *PVALB*, and *α2M,* q-PCR analysis showed two-fold, two-fold, and four-fold significant under-expressions in the liver tissue, respectively. Similarly, significantly down-regulation ($p<0.05$) was observed for *Nattectin* and *PVALB* by three-fold and two-fold, except for *α2M* with no significant difference in expression in spleen sample. Again, in blood samples *Nattectin* was significantly decreased by four-fold, *PVALB* by two-fold, and *α2M* by three-fold in infection-resistant samples. However, these results did not replicate data obtained from an earlier study which used proteomic method. Nevertheless, this study demonstrated that Nattectin, *PVALB*, and *α2M* were over-expressed in infection-susceptible fish providing evidence on the involvement of these gene during *V. parahaemolyticus* infection. These data will provide the foundation for use of biomarkers in identification of disease resistant groupers and selective breeding.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGESANAN BIOPENANDA RENTAN JANGKITAN DARIPADA *Vibrio parahaemolyticus* TERHADAP KERAPU HARIMAU (*Epinephelus fuscoguttatus*) MENGGUNAKAN KAEDAH PCR KUANTITATIF MASA NYATA

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Spesis kerapu termasuk *Epinephelus fuscoguttatus* yang dikenali sebagai kerapu harimau merupakan salah satu spesis bernilai ekonomi yang besar di negara-negara Asia Tenggara. Pelbagai jenis penyakit berjangkit berlaku dalam akuakultur kerapu dan sering mencetuskan masalah dan membawa kepada kerugian ekonomi. Agen penyebab vibriosis dalam akuakultur termasuklah *Vibrio parahaemolyticus*. *V. parahaemolyticus* adalah patogen penyakit bawaan makanan utama dan diklasifikasikan sebagai bakteria gram-negatif halofilik yang biasanya hidup di persekitaran marin dan lautan dan menyebabkan konsumsi masalah kesihatan di seluruh dunia. Selama berabad-abad para petani telah menyedari ciri-ciri fenotip tertentu dalam tumbuh-tumbuhan dan haiwan yang memberikan kelebihan penghasilan mutu satu spesis berbanding yang lain. Kemajuan dalam teknologi telah membolehkan para penyelidik memeriksa ciri-ciri ini di peringkat genetik dan molekul. Pembiakan selektif adalah salah satu pilihan untuk meningkat kesihatan ikan dalam kultur. Kaedah yang pantas dan sesuai untuk pengenalpastian biopenanda yang relevan dalam kumpulan kerapu adalah faktor yang akan membantu dalam pengurusan hasilnya. Kaedah berasaskan qPCR adalah mudah, sesuai digunakan dalam pemeriksaan epidemiologi, genetik dan pengesanan rutin sampel yang banyak dan juga pengekspresan gen biopenanda secara kuantitatif dalam organ-organ dan tisu kerapu. Tujuan kajian ini adalah untuk mengesan biopenanda yang telah dikenal pasti dalam kajian sebelum ini dalam pemilihan ikan dengan kelebihan dalam pertahanan penyakit. Di sini biopenanda berpotensi, alpha-2-Macroglobulin (*α2M*), Parvalbumin Beta-2 Subunit 1 (*PVALB*) dan gen Nattetin telah dipilih. Primer direka bentuk menggunakan Primer-BLAST dari laman web NCBI dan disintesis oleh syarikat tempatan. β-actin (*ACTB*), bertindak sebagai gen rujukan. Jangkitan eksperimental kerapu dengan *V. parahaemolyticus* dijalankan dan tahap pengekpresan gen-gen ini dinilai dalam ikan yang dijangkiti berbanding ikan yang tidak dijangkiti. Ikan kerapu juvenil yang dijangkiti *V. parahaemolyticus* telah dipantau untuk dianalisis rangsangan gen pada tisu sampel yang dikumpulkan selepas 2 minggu. Kerapu yang dijangkiti *Vibrio parahaemolyticus*

menunjukkan kesan peningkatan yang nyata untuk alpha-2-Macroglobulin (*α2M*), Parvalbumin Beta-2 Subunit 1 (*PVALB*), dan Nattectin dalam darah, hati dan limpa berbanding sebelum jangkitan. Pembezaan jangkitan ikan yang mudah-dijangkiti adalah berdasarkan perkembangan saiz lesi kulit lebih daripada lima milimeter (> 5mm) diameter manakala ikan yang tiada sebarang lesi dikenal pasti sebagai ikan rentan jangkitan. Dalam ikan rentan-infeksi, analisa q-PCR mendapati ketiga-tiga gen ini, Nattectin, PVALB, dan α2M, menunjukkan penurunan dua kali ganda, dua kali ganda dan empat kali ganda ekspresi dalam tisu hati, masing-masing berbanding ikan yang diinfeksi. Begitu juga penurunan tahap yang berkesan ($p < 0.05$) diperhatikan dalam Nattectin dan *PVALB* sebanyak tiga kali ganda dan dua kali ganda, masing-masing kecuali *α2M* dimana dalam sampel limpa. Dalam sampel darah pula, Nattectin berkurangan dengan ketara sebanyak empat kali ganda, *PVALB* dua kali ganda, dan *α2M* sebanyak tiga kali ganda dalam sampel dari ikan yang tahan terhadap jangkitan. Tetapi, keputusan ini tidak bersamaan dengan hasil yang diperoleh daripada kajian awal yang sama yang menggunakan kaedah proteomik. Walau bagaimanapun, kajian ini telah mendemonstrasikan kemampuan Nattectin, *PVALB*, dan *α2M* untuk digunakan sebagai biopenanda dalam membezakan ikan yang mudah dijangkiti *V. parahaemolyticus*. Data-data ini akan menyediakan asas untuk penggunaan biopenanda dalam mengenal pasti kumpulan kerapu yang rentan-infeksi dan pembiakan selektif.

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

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Introduction

Groupers are one of the highly desirable species that have high market demand from the family Serranidae. Groupers have superior market value compared to other marine fish (Nurdalila et al., 2015). Due to its economic value, the grouper is commonly cultured by the farming community as well as caught by the fishers from the ocean (Chen et al., 2012).

However, tiger grouper cultivation is plagued with increased prevalence of diseases associated with intensive farming systems and has hampered the development of its culture (Tahir et al., 2018) and become a major constraint to sustainable growth production (Kevin et al., 2015). The decline in growth production is also due to the increasing number of diseases triggered by stress conditions associated with improper intensification systems of farming (Terceti et al., 2016 and Haenen et al., 2014). Poor farming conditions increases the fish susceptibility to opportunistic pathogen across all developmental stages of grouper (Abdullah et al., 2017). A major constraint in grouper hatchery production and farming is vibriosis with approximately 66.7% disease incidence reported and is responsible for up to 50% economic loss globally (Galil et al., 2012 and Chong et al., 2017). *Vibrio parahaemolyticus*, is the most important pathogen that affects aquafarming species (Amalina et al., 2017) due to its pathogenicity and the ability to produce hemolysins that causes severe hemorrhagic septicemia leading to mortality in fish (Noriea et al., 2010) and gastroenteritis syndrome (Harikrishnan et al., 2011). Manifestation of *V. parahemolyticus* infection is often marked with presence of visible red spots on the fish skin, prior its progression towards circular- and/or oval-shaped hemorrhagic ulcer (Hazeri et al., 2016).

This study aimed to continue the work on the selected biomarkers of tiger grouper (*Epinephelus fuscoguttatus sp*) for their response to the bacterial pathogen. Earlier work presented preliminary results on serum proteins that showed a significant increase in *Epinephelus fuscoguttatus sp* disease resistance against *V. parahaemolyticus* (Low et al., 2015). A key research needs to continue the earlier work by Low to identify disease resistant biomarkers and its detection in line with the need to advance mariculture technology and to provide services to farmers in disease management and enhancement of their fish production. Thus, the present study will focus on quantitative PCR-based detection method, which has the capacity for rapid detection and quantitation of gene expressions in association with the identification of disease resistance biomarkers.

1.2 General objective

To identify biomarkers to select infection-resistant grouper.

1.2.1 Specific objectives

1.2.1 To establish real time-PCR-based method to detect gene expressions of selected biomarkers in grouper (*Epinephelus fuscoguttatus*) tissues (blood, spleen, and liver).

1.2.2 To determine the level of expressions of Alpha-2-macroglobulin *(α2M)*, Parvalbumin *(PVALB)* and Nattectin genes in tissues of experimental *Vibrio parahaemolyticus* infected grouper.

1.3 Problem Statement

The rapid development of intensive culture of grouper has resulted in the frequent outbreak of the infectious diseases. The diseases in the grouper contribute to severe economic losses. To overcome these issues, most of the farmers have been using antibiotics and pesticides frequently in their farms to control the diseases and kills the insects or weeds. However, these lead to antibiotic resistant bacteria, which are becoming more and more difficult to treat as we run out of new antibiotics. Furthermore, the use of antibiotics and pesticides in fish farming may affect immune suppression and result in a negative impact to the environment by contaminating the water sediments. In addition, the use of antibiotics and pesticides can be toxic to a host of other organisms and consumers may be ingesting harmful levels of antibiotic residues.

Selection for natural traits with agricultural advantage has been done since humans started farming. With advances in molecular technology, the use of biomarkers to identify disease resistant candidates will be more specific and reproducible and may provide a safer alternative. Study of Low et al, (2014) has revealed the finding of several potential biomarkers in disease resistant Tiger grouper infected with *Vibrio parahaemolyticus* and the results were achieved from comparison of the serum proteome profiles between resistant and susceptible candidates' fish via two- dimensional gel electrophoresis (2-DE) LC/MS/MS. Further analysis is needed to confirm these potential biomarkers as diseases resistant for tiger grouper fish. The Real -time Polymerase Chain Reaction (qPCR) was selected to identify those genes as it is relatively cheaper, rapid, convenient, easy to screen for epidemiological, genetic studies and applicable to routine high- throughput detection of large numbers of sample (Chen et al., 2017). The precise confirmation can be obtained via real-time PCR by specifically identify the genes and differentiate experiment within those susceptible and resistant.

1.4 Research Question

1.4.1 What are the potential biomarkers useful to select for infection-resistant grouper?

1.4.2 Is quantitative-PCR a suitable method to detect these biomarkers?

1.5 Hypothesis

1.5.1 The selected biomarkers are differentially expressed in infection-resistant and infection-susceptible grouper.

1.5.2 The biomarkers are detectable in tissues of grouper using quantitative polymerase chain method (qPCR).

REFERENCES

Abdullah A., Ramli, A., Rimatulhana, Ridzuan, M., Mohammad, M., & Munira. (2017). The presence of Vibrionaceae, Betanodavirus and Iridovirus in marine cage-cultured fish: Role of fish size, water physicochemical parameters and relationships among the pathogens. *Aquaculture Reports*, 7, 56-57.

AFS (Annual Fisheries Statistic). (2014). Annual Fisheries Statistic. Department of Fisheries, Ministry of Agriculture & Agro-Based Industry, Malaysia.

Amalina, Z., M. Z. Dzarifah, Y. Mohd- Termizi, M. N. A. Amal, M. Zamri- Saad, and Ina- Salwany, M.Y. (2017). Phenotypic and genotypic characterization of Vibrio species isolates from Epinephelus species in Selangor, Malaysia. Proceedings of the International Conference on Advances in Fish Health, April 4–6, 2017. Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

Amar, A. M. J. S., Amar, E. C., Faisan, Jr. J. P., Pakingking, Jr. R. V., Satoh, S. (2012). Dietary onion and ginger enhance growth, hemato-immunological responses, and disease resistance in brown-marbled grouper, *Epinephelus fuscoguttatus*. *AACL Bioflux* 5, 231-239.

Andayani, S. R. I. (2011). Immunomodulatory Effect of Alkaloid Bioactive Substances of Jellyfish (*Bougainvillia sp*) on Tiger Grouper *(Epinephelus fuscoguttatus*) *Innate Immune Response*, 9, 203–207.

Aguilera- Rivera, D., Rodríguez- Fuentes, G., Escalante- Herrera, K., Guerra- Castro, E., Prieto- Davó, A., & Gaxiola, G. (2019). Differential expression of immune- related genes in Pacific white shrimp, Litopenaeus vannamei, previously reared in biofloc and challenged with Vibrio harveyi. *Aquaculture Research.* doi:10.1111/are.14063.

Aoki T., Takano T., Santos MD, Kondo H & Hirono I. (2008). Molecular innate immunity in teleost fish: review and future perspectives. Fisheries for Global Welfare and Environment, 5th World Fisheries Congress, 263–276.

Armstrong, P. B., Quigley, J. P. (1999): α-2-Macroglobulin: an evolutionarily conserved arm of the innate immune system. *Developmental and Comparative Immunology*, 23, 375–390.

Arif, S. H. (2008). A Ca2+-binding protein with numerous roles and uses: Parvalbumin in Molecular Biology and Physiology. *Biology Essays* 31, 410-421.

Buckingham, L., and Maribeth, L. F. (2007). Molecular Diagnostics: *Fundamentals, Methods, & Clinical Applications*, 462.

Bunlipaton, P., & Kittichon U. (2017). Growth performance and diseases resistance against *Vibrio vulnificus* infection of novel hybrid grouper (*Epinephelus lanceolatus* x *Epinephelus fuscoguttatus*). *Aquaculture Research*, 48, 1711-1723.

Brooks, G. (1998). Biotechnology in Healthcare: *An Introduction to Biopharmaceuticals, Pharmaceutical Press*, London, UK. (2), 189-219.

Byadgi, O., Chen, Y.-C., Maekawa, S., Wang, P.-C., & Chen, S.-C. (2018). Immune-Related Functional Differential Gene Expression in Koi Carp (Cyprinus carpio) after Challenge with Aeromonas sobria. *International Journal of Molecular Sciences*, 19(7), 2107. doi:10.3390/ijms19072107

Cates, M. S., Teodoro, M. L. & Phillips, G. N. Jr. (2002). Molecular mechanisms of calcium and magnesium binding to parvalbumin. *Biophysical Journal*, 82, 1133–1146.

Cesta, M. F. (2006), Normal structure, function, and histology of the spleen. *Toxicology Pathology*, *34*, (5), 455-465.

Chabanet, P., Bigot, L., Nicet, J. B., Durville, P., Massé, L., Mulochau, T., Obura, D. (2016). Coral reef monitoring in the Iles Eparses, Mozambique Channel (2011-2013). *Acta Oecologica*, 72, 62–71.

Chen J., Li C., Huang R., Du F.K., Liao L.J., Zhu Z.Y. & Wang Y.P. (2012). Transcriptome analysis of head kidney in grass carp and discovery of immune related genes. *BMC Veterinary Research*, *8*, 108–118.

Chevassus, B., Dorson, M. (1990). Genetics of diseases resistant to the fishes. *Aquaculture*. Volume 85, 83-107.

Christine, V. & Edward, M. M. (2012). Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nature Reviews Genetics*. vol 13, 227– 232.

Chong, V.C., Lee, P. K. Y., & Lau, C.M. (2017). Diversity, extinction risk and conservation of Malaysian fishes. *Journal of Fish Biology*. (76), 2009–2066.

Chuang, W., H., Liu, P., C., Hung, C., Y., & Lee, K., K. (2014). Purification, characterization and molecular cloning of alpha-2-macroglobulin in cobia, *Rachycentron canadum*, *Fish Shellfish Immunology.* (41), 346–355.

Chiu, S.T., Tsai, R.T., Jung, P.H., Liu, C.H., & Cheng,W. (2008). Dietary sodium alginate administration to enhance the non-specific immune responses, and disease resistance of the juvenile grouper *Epinephelus fuscoguttatus*. *Aquaculture*. 66-72.

Chuang, W.H., Lee, K.K., Liu, P.C., (2013). Characterization of alpha-2-macroglobulin from groupers. *Fish Shellfish Immunology*.1–10.

Dalia, F. O., Maria, F., Santiago, F.G., Krzysztof Ł. R., Andrzej P., Geert F.W. (2008). Differential transcription of multiple forms of alpha-2-macroglobulin in carp *(Cyprinus carpio)* infected with parasites. *Developmental and Comparative Immunology*, 32, 339–347.

De, M., Mazlan, A.G,Yosni, B., Zaidi, C.C & Simon, K. (2016). Optimum Temperature for the Growth Form of Tiger Grouper (*Epinephelus fuscoguttatus* ♀) × Giant Grouper (*E. lanceolatus* ♂) Hybrid. *Sains Malaysiana. 45*, (4), 541–549.

Ding, X., Lu, D. Q., Hou, Q. H., Li, S. S., Liu, X. C., Zhang, Y., et al. (2012). Orange-spotted grouper (*Epinephelus coioides*) toll-like receptor 22: Molecular characterization, expression pattern and pertinent signalling pathways. *Fish & Shellfish Immunology*, 33(3), 494-503.

FAO. (2018). *The State of World Fisheries and Aquaculture*. 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.

Fahmeeda, M., J., Wan, M., A., Hoe, H., G., Chen, F., L., Syarul, N., B. (2018). Transcriptome data of *Epinephelus fuscoguttatus* infected *Vibrio vulnificus. Data in Briefing,16,*466- 469.

Freedman, S., J., (1991). The role of alpha2-macroglobulin in furunculosis: a comparison of rainbow trout and brook trout. *Comprehension Biochemical Physiology* (98), 549–53.

Funkenstein, B., Rebhan, Y., Dyman, A., & Radaelli, G. (2005). α2-Macroglobulin in the marine fish Sparus aurata. Comparative Biochemistry and Physiology Part A: *Molecular & Integrative Physiology*, 141(4),440–449. doi:10.1016/j.cbpb.2005.06.010.

Galil, A., A. M., Shawky, M. A., (2011). Trials for the control of trichodinosis and gyrodactylosisin hatchery reared *Oreochromis niloticus* fries by using garlic. *Veterinary Parasitology*. (185), 57–63.

Gao, S., Zhao, N., Amer, S., Qian, M., Zhao, B. (2013). Protective efficacy of PLGA microspheres loaded with divalent DNA vaccine encoding the *ompA* gene of *Aeromonas veronii* and the hly gene of *Aeromonas hydrophila* in mice. *Vaccine*, (310), 5754–5759.

Gomez-Gil B, Soto-Rodríguez S, Lozano R, Betancourt-Lozano M (2014). Draft genome sequence of *Vibrio parahaemolyticus* Strain M0605, which causes severe mortalities of shrimps in Mexico. *Genome Announcement*, *2*(2),55-14.

Giglio, V., J., Bender, M., G., Zapelini, C., & Ferreira, C., E., L. (2017). The end of the line. Rapid depletion of a large-sized grouper through spearfishing in a subtropical marginal reef. *Perspectives in Ecology and Conservation*, 15(2), 115–118.

Haenen, O.L.M., Fouz, B., Amaro, C., Isern, M.M., Mikkelsen, H., Travers, M.A., Renault, T., Wardle, R., Hellström, A., Dalsgaard, I., 2014. Vibriosis in aquaculture, (16th ed.) EAFP Conference, Tampere, Finland. 4th September 2013, Bull. Europe. Association. *Fish Pathology*, 34,138–148.

Harikrishnan, R., Balasundaram, C., Moon-Soo, H. S. H. (2011). Dietenriched with mushroom *Phellinus linteusextractenhances* the growth, innate immune response and disease resistance of kelp grouper, *Epinephelusbruneus againstvibriosis*. *Fish Shellfish Immunology*, 30,128-134.

Harikrishnan, R., Balasundaram, C. & Heo, M., S. (2011). Fish health aspects in grouper aquaculture. *Aquaculture*, 320(1-2), 1–21.

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

Hazeri, M., Hassan, M. D., Abba, Y., Omar, A. R., Vishkaei, M. S. (2016). Histopathological evaluation and molecular detection of natural Iridovirus infection in cultured grouper fish in Malaysia. *Complement Clinical Pathology*. (25), 965-971.

Hirono I., Takami, M., Miyata, M., Miyazaki, T., Han, Hj., Takano, T., Endo, M., & Aoki, T., (2004). Characterization of gene structure and expression of two toll-like receptors from Japanese flounder, *Paralichthys olivaceus'*, *Immunogenetics*, 56, 38–46.

Hook, S. E, Gallagher, E. P., Batley, G. E. (2014). The Role of Biomarkers in the Assessment of Aquatic Ecosystem Health. *Integration Environment Assess Manage*. (10),327–41.

Ho, P. Y, Cheng, C. H. & Cheng, W. (2009). Identification and cloning of the α2-macroglobulin of giant freshwater prawn Macrobrachium rosenbergii and its expression in relation with the molt stage and bacteria injection. *Fish & shellfish immunology*, *26*, 459–466. doi.org/10.1016/j.fsi.2009.01.007

Kalic, T., Francoise, M. C., Christian, R., Thimo, R., Aya, T. C., Ines, S., Christiane, H., Annette, K. (2019). *The Journal of Allergy and Clinical Immunology*: In Practise. Vol 7,500-508.

Kevin, D. L., Harvell, C. D., Jon, M. C., Carolyn, S. F., &Sonja, M. S. (2015). Infectious Diseases Affect Marine Fisheries and Aquaculture Economics, *Annual Review of Marine Science,* (7)1,471-496.

Komegae, E. N., Ramos, A. D., Oliveira, A. K., de Toledo S., S. M., Lopes-Ferreira, M. & Lima, C. (2011). Insights into the local pathogenesis induced by fish toxins: Role of natterins and nattectin in the disruption of cell–cell and cell–extracellular matrix interactions and modulation of cell migration. *Toxiconology,* 58(6-7),509–517.

Krasnov, A., Skugor, S., Todorcevic, M., Glover, K. A., & Nilsen, F. (2012). Gene expression in Atlantic salmon skin in response to infection with the parasitic copepod *Lepeophtheirus salmonis*, cortisol implant, and their combination. *BMC Genomics*, 13(1), 130.

Kreuzer, K. A., Lass, U., Landt, O., Nitsche, A., Laser, J., Ellerbrok, H., Pauli, G., Huhn, D. & Schmidt, C. A. (1999). Highly sensitive and specific fluorescence reverse transcription-PCR assay for the pseudogene-free detection of beta-actin transcripts as quantitative reference. *Clinical Chemistry*, 45, 297–300.

Kroon, F., Streten, C., & Harries, S. (2017). A protocol for identifying suitable biomarkers to assess fish health: A systematic review.,12(4),174762. doi:10.1371/journal.pone.

Kumar, S., Raman, R. P., Prasad, K. P., Srivastva, P. P., and Rajendran, K. V. (2017). Modulation of innate immune responses and induction of oxidative stress biomarkers in *Pangasianodon hypophthalamus* following an experimental infection with *dactylogyrid monogeneans*, *Fish and Shellfish Immunology*, (17), 46-48.

Lovell, C. R. (2017). Ecological fitness and virulence features of *Vibrio parahaemolyticus* in estuarine environments. *Application Microbiology Biotechnology*, 101,1781-1794.

Lee, K. K., Liu, P. C., & Chuang, W. H. (2002). Pathogenesis of gastroenteritis caused by *Vibrio carchariae* in cultured marine fish. *Marine Biotechnology*, 4, 267–277.

Li, H., Ye, M. Z., Peng, B., Wu, H. K., Xu, C. X., Xiong, X. P., Wang, C., Wang, S. C., & Peng, X., X. (2010). Immunoproteomic identification of polyvalent vaccines, candidates from *Vibrio parahaemolyticus* outer membrane proteins, Journal Proteome Reserach. (9), 2573–2583.

Lili, W., Yi, L., Shengwei, Z., Huadong, W., Jiming, R., Qiwang, Z. (2018). Transcriptome analysis of grass carp provides insights into the immune-related genes and pathways in response to MC-LR induction. *Aquaculture.* (488), 207-216.

Lieske & Myers. (2009). Coral reef fishes, Princeton University Press, ISBN 9780691089959. http://www.fishbase.org/summary/4460 retrieved on 20 September 2018.

Liu, S. Yu, Z. Zhou, Z. Geoff, W. Sun, F. Lu, J. Zhang, J. Jiang, Y. Hao, Z. Wang, X. (2012). Efficient assembly and annotation of the transcriptome of catfish by RNA Seq analysis of a doubled haploid homozygote, *BMC Genomic*. 13 (1), 595.

Lio-Po, G. D., Lavilla, C. R., & Cruz-Lacierda, E. R. (2001). Health management in aquaculture. Tigbauan, Iloilo, Philippines: *Aquaculture Department, Southeast Asian Fisheries Development Center.* 83-170.

Lopes, F.M., Magalhaes, G.S., Fernandez, I.L.M.J.A., Maria, A.S. (2011). Structural and biological characterization of Nattectin, a new C-type lectin from the venomous fish Thalassophryne nattereri. *Biochimie*. 93, 971-980.

Low C.F., Mariana N.S., Chee H.Y., Aliyu-Paiko M. &Idrus E.S. (2014). Putative apolipoprotein A-I, natural killer cell enhancement factor and lysozyme g are involved in the early immune response of brown-marbled grouper, *Epinephelus fuscoguttatus*, Forskal, to *Vibrio alginolyticus*. *Journal of Fish Diseases* 37, 693–701.

Low, C.-F., Rozaini, M. Z. H., Musa, N. & Syarul Nataqain, B. (2017). Current knowledge of metabolomic approach in infectious fish disease studies. *Journal of Fish Diseases*, 40(10), 1267–1277.doi:10.1111/jfd.12610.

Luo, T., Li, F., Lei, K., & Xu, X. (2007). Genomic organization, promoter characterization and expression profiles of an antiviral gene PmAV from the shrimp *Penaeus monodon. Molecular Immunology*,44,1516–1523.

Ma, H., Mai, K., Xu, W., & Liufu, Z. (2005). Molecular cloning of alpha2-macroglobulin in sea scallop Chlamys farreri (*Bivalvia, Mollusca*). *Fish Shellfish Immuno*logy. *18*(4),345.

Ma, Y., M., Yang, M. J., Wang, S., Li, H. & Peng, X. X. (2015) Liver functional metabolomic discloses an action of L-leucine against Streptococcus iniae infection in tilapias. *Fish & Shellfish Immunology*, (45), 414–421.

Magnadottir, B. (2010). Immunological control of fish diseases. *Marine Biotechnology*. 2, 361–379.

Magnadottir, B. (2006). Innate immunity of fish (overview). *Fish and Shellfish Immunology*. 20, 137–151.

Maier, T., Guell, M., Serrano, L. (2009). Correlation of mRNA and protein in complex biological samples. *FEBS*, (583), 3966–3973.

Marte, C. L., & Toledo, J. D. (2015). Marine fish hatchery: developments and future trends. In M. R. R. Romana-Eguia, F. D. Parado-Estepa, N. D. Salayo, & M. J. H. Lebata- Ramos (Eds.), Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia: Challenges in Responsible Production of Aquatic Species: *Proceedings of the International Workshop on Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia 2014 (RESA)*,189-200.

Martin, A. Douglas, D. F., Houlihan, C. J. (2010). Secombes, Starvation alters the liver transcriptome of the innate immune response in Atlantic salmon (*Salmosalar*), *BMC Genomics:* 11, 418.

Martin, S. A. M., Douglas, A., Houlihan, D. F., Secombes, C. J. (2010), Starvation alters the liver transcriptome of the innate immune response in Atlantic salmon (Salmo salar). *BMC Genomics*, 11, 418.

Marudhupandi, T., Kumar, T. T. A., Prakash, S., Balamurugan, J., & Dhayanithi, N. B. (2017). *Vibrio parahaemolyticus* a causative bacterium for tail rot disease in ornamental fish, *Amphiprion sebae*. *Aquaculture Reports*, 8, 39–44.

Matozzo, V., Pagano, M., Spinelli, A., Caicci, F. & Faggio, C. (2016). Pinna nobilis: a big bivalve with big haemocytes. Fish Shellfish Immunology. (55), (529–534).

Medzhitov, R., & Janeway C. A. (2002). Decoding the patterns of self and non-self by the innate immune system, *Science,* (296),298-300.

Medzhitov, R. (2007). Recognition of microorganisms and activation of the immune response. *Nature*, (449), (819-826).

Mekuchi, M., Kenji, S., Tomofumi, Y., Masahiko, K., & Jun, K. (2017). Trans-omics approaches used to characterise fsh nutritional biorhythms in leopard coral grouper (*Plectropomus leopardus*). *Scinetific Reports,* (7) ,9372.

Miles, A. A., Misra, S. S. & Irwin, J. O. (1938). The estimation of the bactericidal power of the blood. *Journal Hygiene*, (38), 732-749.

Mohammad, N., Mustafa, M., Azmal, M. N. A., Saari, N. H. (2019). Environmental Factors Associated with the Presence of Vibrionaceae in Tropical Cage-Cultured Marine Fishes. *Journal of Aquatic Animal Health*. Vol 31, 154-167.

Muktar, Y., Tesfaye, S., Tesfaye, B. (2016) Present Status and Future Prospects of Fish Vaccination: A Review. *Journal of Veterinary Sciences Technology*, 7, 299.

Mustafa, S., Estim, A., Raehanah, S., & Shapawi, R., (2018), Positioning of Aquaculture in Blue Growth and Sustainable Development Goals Through New Knowledge,Ecological Perspectives and Analytical Solutions. *Aquaculture Indonesiana* 19(1),1-9.

Nagasawa, K., & Cruz-Lacierda, E. R. (2004). Diseases of cultured groupers Southeast Asian Fisheries Development Center, *Aquaculture Department.* 227-230.

Nakamoto, M., Takeuchi, Y., Akita, K., Kumagai, R., Suzuki, J., Koyama, T. & Sakamoto, T. (2017). A novel C-type lectin gene is a strong candidate gene for Benedenia disease resistance in Japanese yellowtail, Seriolaquinqueradiata. *Developmental & Comparative Immunology,* 76, 361–369.

Nakao M, Mutsuro J, Nakahara M, Kato Y, Yano T. (2003). Expansion of genes encoding complement components in bony fish: biological implications of the complement diversity. *Development Component Immunology, 27*(9),749–62.

Nair, G. B., Ramamurthy, T., Bhattacharya, S. K., Dutta, B., Takeda, Y., & Sack, D. A., (2007). Global dissemination of *Vibrio parahaemolyticus* serotype O3:K6 and its serovariants. *Clinical Microbiology Review. 20* (1), 39–48.

Noriea, N. F. III, Johnson, C. N., Griffitt, K. J. & Grimes, D. J. (2010). Distribution of type III secretion systems in *Vibrio parahaemolyticus* from the northern Gulf of Mexico. *Journal of Applied Microbiology*, 109, 953–962.

Nurdalila, A. A., Bunawan, H., Kumar, S. V., Rodrigues, K. F., & Baharum, S. N. (2015). Homogeneous Nature of Malaysian Marine Fish *Epinephelus fuscoguttatus* (Perciformes; *Serranidae*): Evidence Based on Molecular Markers, *Morphology and Fourier Transform Infrared Analysis*. 40-56.

Ødegård, J., Baranski, M., Gjerde, B., & Gjedrem, T., (2011). Methodology for genetic evaluation of disease resistance in aquaculture species: challenges and future prospects. *Aquaculture Research*, 42, 103–114.

Oshiumi, H., Matsuo, A., Matsumoto, M. &Seya, T. (2008). Pan-vertebrate Toll-Like receptors during evolution, *Current Genomics*, (9), 48–93.

Palm, N., & Medzhitov, R. (2009). Pattern recognition receptors and control of adaptive immunity, *Immunol Revolution*, 227, 21–33.

Pears, R. J., Choat, J. H., Mapstone, B. D. & Begg, G. A. (2006). Demography of a large grouper *Epinephelus fuscoguttatus*, from Australia's Great barrier reef: implications for fishery management. Marine Ecology Progress Series. (307),259-272.

Peng, B., Ma, Y. M., Zhang J. Y. & Li, H. (2015). Metabolome strategy against *Edwardsiellatarda* infection through glucose enhanced metabolic modulation in tilapias. *Fish & Shellfish Immunology*, 45, 869–876.

Peter, H. & Thrall, 1. J. (2010). Blackwell Publishing Ltd 3 Evolutionary change in agriculture: the past, present. *Evolutionary Applications.* 405–408.

Peng, B., Ma, Y. M., Zhang, J. Y. & Li, H. (2015). Metabolome strategy against *Edwardsiella* tarda infection through glucose enhanced metabolic modulation in tilapias. *Fish & Shellfish Immunology* 45, 869–876.

Pierre, S., Gaillard, S., Prévot- D'Alvise N., Aubert, J., Rostaing- Capaillon, O., & Leung- Tack, D. (2008). Grouper aquaculture: Asian success and Mediterranean trials. *Aquatery Conserve Marine Freshwater Ecosystem.18*(3),297–308.

Preetham, E., Abdul S. R., Jesu A., Ratree, W., Matteo C., Einar, R., & Baskaralingam, V. (2019). The Role of Lectins in Finfish: A Review, *Reviews in Fisheries Science & Aquaculture*, *27*(2),152-169.

Ponprateep, S., Vatanavicharn, T., Lo, C. F., Tassanakajon, A., & Rimphanitchayakit, V. (2017). Alpha-2-macroglobulin is a modulator of prophenoloxidase system in pacific white shrimp Litopenaeus vannamai. *Fish & Shellfish Immunology*, 62, 68–74. doi:10.1016/j.fsi.2016.12.028.

Pu, Y., Zhu, J., Wang, H., Zhang, X. & Chen, D. (2016). Molecular characterization and expression analysis of Hsp90 in *Schizothorax prenanti. Cell Stress and Chaperones.*23-27

Rajan, B., Fernandes, J. M., Caipang, C. M., Kiron, V., Rombout, J. H. & Brinchmann M. F. (2011), Proteome reference map of the skin mucus of Atlantic cod (*Gadus morhua)* revealing immunecompetent molecules. *Fish Shellfish Immunology*, 31, 224- 231.

Rauta, P. R., Nayak, B. & Das, S. (2012). Immune system and immune responses in fish and their role in comparative immunity study: a model for higher organisms, *Immunology Letter*.148. (23–33).

Rimmer, M. A. & Glamuzina, B. (2018). A review of grouper (Family Serranidae; Sub family *Epinephelinae*) Aquaculture from a Sustainability Science Perspective Review. *Aquaculture.* 58-87.

Rees, C. L. (2016). Molecular biomarkers and network analyses of neuron-type connectivity reveal cloaked insights into hippocampal function (Order No.10248990). Available from ProQuest Dissertations & Theses Global.

Robertsen, B., Rokenes, T. P., & Larsen, R. (2007): Atlantic salmon ISG15: expression and conjugation to cellular proteins in response to interferon, double-stranded RNA and virus infections. *Molecular Immunology*, 44, 950–959.

Romero, P., (2002). An etymological dictionary of taxonomy. Madrid, unpublished. https://www.fishbase.de/references. Retrieved on, 31 December 2018.

Rojo-cebreros, A. H., Ibarra-castro, L., & Martínez-brown, J. M. (2018). Fish and Shell fish Immunology Immunostimulation and trained immunity in marine fish larvae. *Fish and Shellfish Immunology*, 80, 15–21.

Saeij, J. P., Vries, B. J., & Wiegertjes, G. F. (2003). The immune response of carp to Trypanoplasma borreli: kinetics of immune gene expression and polyclonal lymphocyte activation. *Development Comparative Immunology*,*27*, (10),859-74.

Sankar, P., A. G., Telang, A. & Manimaran, (2010). Curcumin protects against cypermethrin-induced genotoxicity in rats. *Environment Toxicology Pharmacology*, 30, 289–291.

Schrama, D., Cerqueira, M., Raposo, C. S., Rosa da Costa, A. M., Wulff, T., Gonçalves, A. & Rodrigues, P. M. (2018). Dietary Creatine Supplementation in Gilthead Seabream (Sparus aurata): Comparative Proteomics Analysis on Fish Allergens, Muscle Quality, and Liver. *Frontiers in Physiology*, 9.

Schwanhäusser, B. (2011). Global quantification of mammalian gene expression control. *Nature*,473, 337–342.

Seebacher, F., & Walter, I. (2012). Differences in locomotor performance between individuals: importance of parvalbumin, calcium handling and metabolism. *Journal Experimental Biology*, (215), 663–670.

Shapawi, R., Ebi, I., Yong, A. S. K. & Ng, W. K. (2014). Optimizing the growth performance of brown-marbled grouper, *Epinephelus fuscoguttatus* (Forskal) by varying the proportion of dietary protein and lipid levels. *Animal Feed Science and Technology*, (191),98–105.

Smith, P. (2008). Antimicrobial resistance in aquaculture. Revue Scientifique et Technique. *(International Office of Epizootics*) 27,243–264.

Somboonwiwat, K., Vorrapon, C., Hao-Ching, W., Chu, F. L., & Anchalee T. (2010). Proteomic analysis of differentially expressed proteins in *Penaeus monodon* hemocytes after *Vibrio harveyi* infection. *Proteome Science.* (8), 39.

Soto-Rodriguez, S. A. B., Gomez-Gil, R., Lozano-Olvera, M., Betancourt-Lozano, M. S. & Morales-Covarrubias. (2015). Field and experimental evidence of *Vibrio parahaemolyticus* as the causative agent of acute hepatopancreatic necrosis disease of cultured shrimp *(Litopenaeus vannamei)* in northwestern Mexico. *Application Environment Microbiology*, 81, 1689 – 1699.

Starkey, P. M., Fletcher, T. C. & Barrett, A. J. (1982). Evolution of alpha2-macroglobulin. The purification and characterization of a protein homologous with human alpha2- macroglobulin from plaice *(Pleuronectes platessa L.)* plasma. *Biochemical Journal*, (205), 97–104.

Sun, Y., Guo, C. Y., Wang, D. D., Li, X. F., Xiao, L., Zhang, X. & Zhang, Y. (2016). Transcriptome analysis reveals the molecular mechanisms underlying growth superiority in a novel grouper hybrid (*Epinephelus fuscogutatus*♀ × *E. lanceolatus*♂*). BMC Genetics*, 17,1.

Tahir, D., Shariff, M., Syukri, F., & Yusoff, F. M. (2018). Serum cortisol level and survival rate of juvenile *Epinephelus fuscoguttatus* following exposure to different salinities. *Veterinary world*, *11*(3), 327-331.

Tafalla, C., Figueras, A., Novoa, B. (2001). Viral hemorrhagic septicemia virus alters turbot *Scophthalmus maximus* macrophage nitric oxide production. *Disease of Aquatic Organism*, 47, 101–107.

Tan, S. C., & Yiap, B. C. (2009). DNA, RNA, and protein extraction: the past and the present. *Journal of biomedicine & biotechnology*, 574398.

Tan, X., Sun, Z., Liu, Q. & Lin, H. (2018). Effects of dietary ginkgo biloba leaf extract on growth performance, plasmabiochemical parameters,fish composition, immune responses, liverhistology, and immune and apoptosis-related genes expression of hybrid grouper (*Epinephelus lanceolatus*♂×*Epinephelus fuscoguttatus*♀) fed highlipid diets. *Fish and Shellfish Immunology*, 72,399-409.

Tedeschi, J. N., Kennington, W. J., Berry, O., Whiting, S., Meekan, M., & Mitchell, N. J. (2015). Increased expression of Hsp70 and Hsp90 mRNA as biomarkers of thermal stress in loggerhead turtle embryos (*Caretta Caretta*) *Journal Therminal Biology*, 47, 42-50.

Terceti, M. S., Hamdi, O. & Carlos, R. O. (2016). Photobacterium *damselae subsp. damselae*, an Emerging Fish Pathogen in the Black Sea: Evidence of a Multiclonal Origin. *Applied and Environment Microbiology,* (16),781.

Tesena, P., Korchunjit, W., Taylor, J., & Wongtawan, T. (2017). Comparison of commercial RNA extraction kits and qPCR master mixes for studying gene expression in small biopsy tissue samples from the equine gastric epithelium. *Journal of Equine Science*, *28*(4), 135–141.

Turner, J. W., Malayil, L., Guadagnoli, D., Cole, D., & Lipp, E. K. (2014). Detection of *Vibrio parahaemolyticus, Vibrio vulnificus* and *Vibrio cholerae* with respect to seasonal fluctuations in temperature and plankton abundance. *Environmental Microbiology*, *16*(4), 1019-1028.

Uribe, C., Folch, H., Enriquez, R., & Moran, G. (2011). Innate and adaptive immunity in teleost fish : a review, (10), 486–503.

Xiang, X. W., Xiao, J. X., Zhou, Y. F., Zheng, B., & Wen, Z. S. (2019). Liver transcriptome analysis of the *Sparusmacrocephlus* in response to *Vibrio parahaemolyticus* infection. *Fish & Shellfish Immunology*, (84), 825–833.

Xu, X. J., Sang, B. H., Chen, W. B., Yan, Q. P., Xiong, Z. Y., & Su, J. B. (2015). Intracellular survival of virulence and low-virulence strains of *Vibrio parahaemolyticus* in Epinephelusawoara macrophages and peripheral leukocytes, *Genetic Molecular Resolution,*14,706-718.

Xu, L.W., Liu, X. H., Zhang, J. Y., Liu, G. F., & Feng, J. (2016). Outbreak of enteric microsporidiosis of hatchery-bred juvenile groupers Epinephelus spp., associated with a new intranuclear microporidian in China. *Journal of Fish Diseases*, *40*(2), 183–189. doi:10.1111/jfd.12528.

Wang, L., Jie, Z., Xianghui, K., Xianliang, Z., and Li, L. (2017). A C-type lectin, Nattectin- like protein (CaNTC) in Qihe crucian carp, Carassius auratus: Binding ability with LPS, PGN and various bacteria, and agglutinating activity against bacteria. *Fish & Shellfish Immunology*. (67), 382-392.

Wang, N., Whang, I. & Lee, J. (2008). A novel C-type lectin from abalone, Haliotis discus discus, agglutinates *Vibrio alginolyticus*. *Development Comparative Immunology*,(32), 1034–1040.

Wang, P., Wang, J., Su, Y. Q., Mao, Y., Zhang, J. S., Wu, C. W., Ke, Q. Z., Han, K. H., Zheng, W. Q. & Xu, N. D. (2016). Transcriptome analysis of the *Larimichthys crocea* liver in response to Cryptocaryon irritants. *Fish Shellfish Immunology*, 48, 1–11.

Wassom, D. L. & Kelly, E. A. B. (1990). The role of the major histocompatibility complex in resistance to parasite infections. *Immunology*. Vol.10,31-52.

Wei, J., Xu, D., Zhou, J., Cui, H., Yan, Y., Ouyang, Z., Gong, J., Huang, Y., Huang, X., Qin, Q. (2010). Molecular cloning, characterization and expression analysis of a C-type lectin (Ec-CTL) in orange-spotted grouper, *Epinephelus coioides*. *Fish Shellfish Immunology*. 28,178–186.

Wei, Y. C., Pan, T. S., Chang, M. X., Huang, B., Xu, Z., & Luo, T. R. (2011). Cloning and expression of Toll-like receptors 1 and 2 from a teleost fish, the orange-spotted grouper *Epinephelus coioides*. *Veterinary Immunology and Immunopathology*, 141,173-182.

Whyte, S. K. (2007). The innate immune response of finfish – a review of current knowledge. *Fish Shellfish Immunology,* (23), 1127–1151.

Wilson, W., Lowman, D., Antony, S. P., Puthumana, J., Bright Singh, I. S., & Philip, R. (2015). Immune gene expression profile of Penaeus monodon in response to marine yeast glucan application and white spot syndrome virus challenge. *Fish & Shellfish Immunology,* 43(2), 346–356. doi:10.1016/j.fsi.2014.12.032.

Wolf, J. C., & Wolfe, M. J. (2005). A Brief Overview of Nonneoplastic Hepatic Toxicity in Fish. *Toxicologic Pathology, 33*(1), 75–85.

Yano, T. (1996). The nonspecific immune system: Humoral defence. In: Iwama G, Nakanishi T: *The Fish Immune System:* Organism, Pathogen and Environment. Academic Press, San Diego. 105–157.

Yu, J., Sun, J., Zhao, S., Wang, H., & Zeng, Q. (2019). Transcriptome analysis of oriental river Prawn (*Macrobrachium nipponense*) Hepatopancreas in response to ammonia exposure. *Fish & Shellfish Immunology*. doi:10.1016/j.fsi.2019.07.036.

Zorrilla, I., Arijo, S., Chabrillon, M., Diaz-Rosales, P., Martinez-Manzanares, E., Balebona, M. C. (2004). Vibrio species isolated from diseased farmed sole, *Solea senegalensis* (Kaup), and evaluation of the potential virulence role of their extracellular products. *Journal Fish Disease,* 26,8.

Zhang, C., Zhao, Z., Liu, G. Y., Li, J., Wang, G. X., & Zhu, B. (2018). Immune response and protective effect against spring viremia of carp virus induced by intramuscular vaccination with a SWCNTs-DNA vaccine encoding matrix protein. *Fish & Shellfish Immunology, 79*, 256-264.

Zhao, X. L., Han, Y., Ren, S. T., Ma, Y. M., Li, H., & Peng, X. X. (2015). L-proline increases survival of tilapias infected by *Streptococcus agalactiae* in higher water temperature. *Fish & Shellfish Immunology*,44,33–42.

Zhou, C., Lin, H., Huang, Z., Wang, J., Wang, Y. &Yu, W. (2015). Effects of dietary soybean isoflavones on non-specific immune responses and hepatic antioxidant abilities and mRNA expression of two heat shock proteins (HSPs) in juvenile *golden pompano Trachinotus ovatus* under pH stress. *Fish & shellfish immunology,* 47,1043–1053.

Zhu, K., Zhang, D., Wei, J., Huang, G., Guo, Y., Jiang, S., & Yu, D. (2016). The complete mitochondrial genome of the hybrid grouper *Epinephelus fuscoguttatus* (♀)×Epinephelus lanceolatus (♂), *Mitochondrial DNA Part A*, *27*(3), 1968- 1969.