2015-2016 Sabbatical Research Report:

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Summary: *Thanks so much for awarding me one semester (2015Fall) sabbatical leave for my research during the academic year of 2015-2016. During this period, I conducted my research studies in Fish Disease Lab at Lake Superior State University together with a visiting professor from China, and several LSSU senior students. I finished several projects and finish writing several manuscripts and publishing them in peer-reviewed journals. My students and I also attended several scientific conferences/meeting and presented recent results. Meanwhile, I also visited my collaborator s at University of Pennsylvania, Michigan State University and Institute of Oceanology, Chinese Academy of Sciences to strengthen collaborations for research grants. During my sabbatical leave, I wrote and submitted one proposal for grant and got funded from China National Science Foundation. The details are described as follows.*

1. Research activities:

Project 1: ***Monitoring fish movement and fish condition in tributaries of Whitefish Bay.***
2015-2016. Bureau of Indian Affairs-Great Lakes Restoration Initiative Funds (BIA-GLRIF). $142,964. Co-PI ($ 80,033 for LSSU).

This project was funded from **Bureau of Indian Affairs Great Lakes Restoration Initiative (BIA-GLRI)** for the period of **2015-2016.** Dr. Paul Ripple from **Bay Mills Biological Services** MI, is the PI of the project, Drs. Ashley Moerke, Kevin Kapuscinski, and Jun Li from LSSU are co-PI for the project. Dr. Li is in charge of the analysis of fish health status of the collected fish samples from three tributaries of Whitefish Bay, which was also part of the senior research project of Lucas Bradburn, a senior LSSU fish health major student. For the fish pathogens diagnosis, we proposed to analyze 3 bacteria, and 3 viruses, that are the most popular pathogens in the Great Lakes area. We applied q-ELISA for a quick survey of over 200 fish samples. This is the first research survey about fish health status for the wild fish from UP. The fish samples were collected from April 2015 through November, 2015. Then we continued the laboratory analysis for blood cells, bacteria and virus diagnosis. In our study, we discovered a high percentage of positive fish to VHS, ISA and

BKD in the wild-caught healthy-appearing fish especially Longnose Sucker, Coho salmon. We finished this project. As part of Lucas Bradburn's senior research project, Lucas presented his results in the Biological Senior Research Symposium and Mid-west Fishery and Wildlife Conference. Dr. Li presented this results at the ARL-MDNR joint meeting. Dr. Gao, visiting professor from Shanghai Ocean University, and Jing Zhang, an exchange Chinese student were also involved in the project.

Project 2: ***Effect of Dietary beta-glucan Derived from Algae on Growth Performance, Disease Resistance and Immune Response in Atlantic salmon.*** 2015,1-2015, 12, Contract from *Algal Scientific Company* $20,746. (Dr. Li as PI).

This project was a contract research project between Dr. Li (LSSU) and Algal Scientific Company. We have finished the contract work. In the study, we found beta-glucan derived from algae (product of Algal Scientific Company) can enhance the growth performance of Atlantic salmon fries after feeding for 2 -4 weeks. After challenge with pathogenic bacteria (*Aeromonas salmonicida*), the fish fed with beta-glucan feeds showed great increase of disease resistance in a dose-dependent manner. Dr. Li presented this data in the "*International Workshop on Aquaculture Health*" held in Qingdao, China during this summer. Dr. Gao, and two Chinese exchange students, (Jing Zhang, Hui Zhao) and LSSU fish health major students (Lucas Bradburn, Conner Workentine) were involved in the related research.

Project 3: *Re-Emergence of Epizootic Epitheliotropic Disease Virus: Potential Effects and Development of Improved Diagnostics &Control Measures*. 2015-2016 Funded from Great Lakes Fishery Trust. $446,492. Co-PI. (PI, Dr. Mohamed Faisal from Michigan State University, East Lansing, MI). ($69,454 for LSSU).

This is a collaborative research project together with MSU. Dr. Li worked as co-PI and leader at LSSU. We have finished part of the project, including fish sampling, blood serum preparation, IgM purification from fish blood serum and generation of antibodies. Further studies are still on-going.

Project 4. 2014-2019 Key Project from Chinese Natural Science Fund for the study Immuno-escape Mechanisms of *Edwarsiella tarda* in Turbot. Chinese Yuan (RMB) 3,000,000.00, Co-PI. (PI, Dr. Li Sun, from Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China).

Project 5: 2015-2016 Chinese Natural Science Fund for "The immune mechanism to

generate protective immunity for *Edwardsiella tarda* inactivated vaccine. RMB 270,000. Co-PI, (PI, Dr. Guoshi,Xie Yellow Sea Fishery Research Institute, Qingdao, China).

Both Project 4 and 5 are collaborative research projects between Dr. Li and his collaborators in the institutions in China. Dr. Li went to China during the summer break and conducted such research studies in China for several weeks. All researches goes well. We have published 3 papers in peer-reviewed journals and two manuscripts are under revision in 2016. Both projects are on-going.

In addition, Dr. Li also finished the fish disease diagnostic assays for BKD and EMS which are the routine disease diagnosis contract with Michigan DNR for the MDNR raised fish in the hatcheries. Dr. Li also did a new contract research with Sault Tribe Fisheries for the VHSV diagnostic assay from walleyes.

2. **Research Training for Undergraduates**

During the period of 2015-2016, I have involved a visiting professor from Shanghai Ocean University (Dr. Gao) and several Fish Health and Fisheries students (two exchange students from China and 3 LSSU senior students) in the funded research projects. These research projects provide more hands-on opportunities for the students. All of them received very good trainings in fish sampling, disease diagnosis, hematological and immunological assays, as well as data analysis. Based on the training, the Fish Health students Lucas Bradburn, and Conner Workentine were received LSSU Undergraduate Research Grant for their senior research project. Lucas also awarded the Fund for LSSU in support him to attend scientific conference/meetings to present his research results. Conner and Scott Cooper, both received 2016 summer internship supported by Sea Grant and worked in Michigan fish farms. Their good trainings at LSSU allow them to show very satisfied performance during their Summer Intern. They are good examples to show the key value of LSSU's education mission.

3. **Grants and Contracts**

During the sabbatical leave, Dr. Li wrote an international collaborative grant from Chinese Natural Science Fundation and got funded (1). Dr. Li also generated a new research contract with Sault tribe Fisheries for diagnosis (2).

1) "Involvement of phagocytic B cells in the Defense Mechanism in Marine Fish". 2016, 1-2017,12; RMB200,000. (PI, Dr. Li).

2) 2016, Contract research for diagnosis of VHS virus from walleyes with Sault Ste Marie Tribe Fishery Research Station. $450.

4. **Scientific Conference/meetings:**

1) Bradburn L., Gao JZ. & **Li J.** *2016.* Health Status of Migrating and Resident Fishes in Three Tributaries of Whitefish Bay, Lake Superior. *76th Midwest Fisheries and Wildlife Conference. January 24-26, Grand Rapids, Mi.*

2) **Li J.** *2015, Adjuvant effects of QS saponins in Turbot (Scophthalmus maximus) upon bath vaccination.* The 13th International Society of Developmental and Comparative Immunology Congress. June 28-July 3, 2015. Murcia, Spain.

3) **Li J.** 2016. *Application of Immuno-stimulants in Aquaculture.* International Workshop on Aquaculture Health, July 28-29, Qingdao, China.

4) **Li J.**, Sun L. and Woo NYS. 2016. *Vaccination of Silver sea bream (Sparus sarba) against Vibrio alginolyticus.* In: 2nd International Conference of Fish & Shellfish Immunology". June 26-30, 2016. Portland, ME, USA.

5. **Lists of Publications (with abstract):**

1) Wang L.L., Wang L., H. Zhang, D.X. Zhang, H.Wang, **J. Li,** LS Song. 2015. The multi-component complement system and its potential activation pathway in oyster *Crassostrea gigas.* ***Scientific Reports*** (under revision).

Abstract: The complement system is one of major mechanisms of immune system playing essential roles in innate and adaptive immune response. In the present study, the counterparts of vertebrate key complement components were investigated by screening genomic data of

Crassostrea gigas, and 792 gene models containing complement-related domains were identified. The transcriptomes of haemocytes were surveyed at 6, 12 and 24 h post LPS stimulation, 77 C1q domain containing proteins, 53 C-type lectins and 42 fibrinogen-related proteins were sorted with different expression. There were 18 serine protease domain-containing (SPC) proteins, 4 MACPF-domain containing proteins and 11 C3 receptor-like proteins up-regulated upon LPS stimulation. The CgC3 mRNA was significantly increased at 12 h after LPS stimulation, and the existing pattern of CgC3 in cell free plasma and its capability to process into three subunit chains were confirmed. The complement related PRRs with coiled coil region presumably responsible for recognizing pathogens and SPC proteins with CUB domain may devote to activation of CgC3, following C3-like receptors with intergrin-α/β domain mediating phagocytosis of C3-labled pathogens. Those complement components apparently serve as opsonin for assisting phagocytosis of opsonized pathogens. The overall results emerged from the present study would suggest the existence of a multicomponent system in *C. gigas*.

2) Li M.F., Li J. Sun L., 2016. CsMAP34, a teleost MAP with a dual role: a promoter of complement activation and a regulator of immune cell activity. ***Scientific Reports,*** (under revision).

Abstract: In teleost fish, the immune functions of mannan-binding lectin (MBL) associated protein (MAP) and MBL associated serine protease (MASP) are scarcely investigated. In the present study, we examined the biological properties of both MAP (CsMAP34) and MASP (CsMASP1) molecules from tongue sole (*Cynoglossus semilaevis*). We found that CsMAP34 and CsMASP1 expressions occurred in nine different tissues and were upregulated by bacterial challenge. CsMAP34 expression was detected in blood, especially during bacterial infection. Recombinant CsMAP34 (rCsMAP34) could bind with tongue sole MBL (rCsBML) when the latter was activated by bacteria, while recombinant CsMASP1 (rCsMASP1) bound activated rCsBML only in the presence of rCsMAP34. rCsMAP34 stimulated the hemolytic and bactericidal activities of serum complement, whereas CsMAP34 antibody blocked these activities of the complement. Knockdown of CsMASP1 in tongue sole resulted in significant inhibition of complement activities. Furthermore, rCsMAP34 interacted directly with peripheral blood leukocytes (PBL) and could enhance the respiratory burst, acid phosphatase activity, chemotactic activity, and gene expression of PBL. These results indicate for the first time that CsMAP34 acts, on one hand, a regulator that promotes the lectin pathway of complement activation via its ability to recruit MBL to MASP, and, on the other hand, a modulator of immune cell activity.

3) Sun Y., Liu L., **Li J.** and Sun L. 2016. Three novel B-type mannose-specific lectins of *Cynoglossus semilaevis* possess varied antibacterial activities against Gram-negative and Gram-positive bacteria. ***Developmental and Comparative Immunology, 55:194-202.***

Abstract: Lectins are a group of sugar-binding proteins that are important factors of the innate immune system. In this study, we examined, in a comparative manner, the expression and function of three Bulb-type (B-type) mannose-specific lectins (named CsBML1,

CsBML2, and CsBML3) from tongue sole. All three lectins possess three repeats of the conserved mannose binding motif QXDXNXVXY. Expression of CsBML1, CsBML2, and CsBML3 was most abundant in liver and upregulated by bacterial infection. Recombinant (r) CsBML1, CsBML2, and CsBML3 bound to a wide arrange of bacteria in a dose-dependent manner and with different affinities. All three lectins displayed mannose-specific and calcium-dependent agglutinating capacities but differed in agglutinating profiles. rCsBML1 and rCsBML2, but not rCsBML3, killed target bacteria in vitro and inhibited bacterial dissemination in fish tissues in vivo. These results indicate for the first time that in teleost, different members of B-type mannose-specific lectins likely play different roles in antibacterial immunity.

4) Wang YJ, Wang XH , Huang J, & **Li J***. 2016. Adjuvant Effect of *Quillaja Saponaria* Saponin (QSS) In Turbot (*Scophthalmus Maximus*) Upon Bath Vaccination. ***International Journal of Molecular Sciences***, *17*(3), 325; doi:10.3390/ijms17030325.

Abstract: The adjuvant effect of *Quillaja saponaria* saponin (QSS) on protection of turbot fry was investigated with immersion vaccination of formalin-killed *Vibrio anguillarum* O1 and various concentrations of QSS (5, 25, 45 and 65 mg/L). Fish were challenged at days 7, 14 and 28 post-vaccination. Significantly high relative percent of survival (RPS) ((59.1 ˇ 13.6)%, (81.7 ˇ 8.2)%, (77.8 ˇ 9.6)%) were recorded in the fish that received bacterins immersion with QSS at 45 mg/L, which is comparable to the positive control group vaccinated by intraperitoneal injection (IP). Moreover, a remarkably higher serum antibody titer was also demonstrated after 28 days in the vaccinated fish with QSS (45 mg/L) than those vaccinated fish without QSS ($p < 0.05$), but lower than the IP immunized fish ($p < 0.05$). Significant upregulation of IgM gene expression has also been identified in the tissues of skin, gill, spleen and kidney from the immunized fish in comparison to the control fish. Taken together, the present study indicated that QSS was able to dramatically evoke systemic and mucosal immune responses in immunized fish. Therefore, QSS might be a promising adjuvant candidate for fish vaccination via an immersion administering route.

5) **Li J***. Ma S.Y., Woo NYS. 2016. Vaccination of silver sea bream (*Sparus sarba*) against *Vibrio alginolyticus*: protective evaluation of different vaccinating modalities. ***International Journal of Molecular Sciences***, *17*(1), 40; doi:10.3390/ijms17010040

Abstract: In order to develop more effective immunological strategies to prevent vibriosis of farmed marine fish in Hong Kong and southern China, various vaccine preparations including formalin-,phenol-, chloroform- and heat-killed whole cell bacterins and subcellular lipopolysaccharides (LPS),as well as different administration routes, were investigated. Fish immunized with the subcellular LPS exhibited the best protection [Relative Percent of Survival (RPS) = 100], while fish immunized with whole cell bacterins displayed varying

degrees of protection (RPS ranged from 28 to 80), in descending order: formalin-killed > phenol-killed > heat-killed > chloroform-killed bacterins. Regarding various administration routes, fish immunized with two intraperitoneal (i.p.) injections exhibited the best protection, and the RPS values were 100 or 85 upon higher or lower doses of pathogenic *V. alginolyticus* challenges. Both oral vaccination and a combination of injection/immersion trial were also effective, which achieved relatively high protection (the RPS values ranged from 45 to 64.3). However, two hyperosmotic immersions could not confer satisfactory protection, especially when fish were exposed to the severe pathogenic bacteria challenge. Marked elevations of serum agglutinating antibody titer were detected in all immunized fish. Macrophage phagocytosis was enhanced significantly, especially in the fish immunized by formalin- and phenol-killed bacterins through various administration routes. Both adaptive (specific antibody) and innate (phagocytic activity) immunity elicited by different immunization strategies were in parallel with the degree of protection offered by each of them. Although all vaccination trials had no significant effect on the serum hematocrit and hemoglobin levels, the circulating lymphocyte counts were significantly elevated in the fish immunized with LPS, formalin- and phenol-killed bacterins. Serum cortisol levels appeared to be reduced in all immunized fish except the trial of hyperosmotic immersion, which indicated the stressful impact on vaccinated fish.