Aims

This study aims to evaluate the gene expression of CYP 1A1, 1B1, and 3A of the Nile tilapia from the Philippines. This study will determine the liver mRNA expression of selected CYPs as toxicity or stress biomarkers.

Procedure

Sample collection

Nile tilapias were collected from February 22 – March 4, 2013 in Laguna Lake (Sta. Rosa and Los Banos sites), and in freshwater fisheries in Bilar, Bohol, Philippines. The fish samples were immediately dissected on board after measurement of biometry (body length, body weight, etc.). Organ (liver, spleen, muscle, and kidney) samples were removed and total organ weight was measured. The subsamples were frozen in liquid nitrogen, transported to Ehime University, Japan, and stored at -80°C.

Quantitative PCR

To quantify mRNA expression levels of different CYPs, two step real-time RT-PCR was performed. Total RNA were isolated from the liver samples in each sampling sites and then treated with DNase. After reverse transcription by a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems), the cDNA were applied for a real-time PCR using SYBR® Premix Ex Taq TM (Tli RNaseH Plus) Master Mix and analyzed using StepOnePlusTM Software (Applied Biosystems). The optimization of the real-time PCR reaction was performed according to the manufacturer's instructions (Applied Biosystems). The PCR conditions were 10 min at 95 °C, 40 cycles of 15 s at 95 °C and 60 s at 95 °C. The calibration curves were generated by plotting Ct values against logarithmic value of template concentration. Each reaction was run in triplicate and normalization was performed.

RESULTS AND DISCUSSION

Laguna Lake is among the largest and one of the economically important water system in the Philippines. It is the largest lake in the Philippines and ranked as the 3rd largest in the whole of South East Asia and that twenty- two rivers and streams gutter into the lake (Laguna Lake Development Authority, 2016). While the bay provided a lot of resources in the country, it is continuously endangered by pollutants.

Our previous study (LaMer Project 28-08, 2016) showed that cytochrome P450 content of the Nile tilapia from two sampling sites in Laguna lake and one sampling site in Bilar, Bohol ranged from 223.988 to 297.641 pmol CYP450/ mg microsome. In various studies, high CYP450 content had been highly correlated with the occurrence of pollutants in the environment (Bainy et al. 1999; Chen et al. 2001). CYP450 is the major biochemical marker in pollution studies as its expression is induced by the presence of endogenous and exogenous chemicals and drugs.

To confirm the results of our previous study, gene expression of CYP 1A1, 1B1, and 3A was performed.

RNA Isolation and Concentration

Total RNA were isolated from the liver samples in each sampling sites and the concentration of each was determined using NanoDrop® ND-1000 Spectrophotometer. The RNA concentrations ranged from 447.09 to 2412.86 ng/ul (Table 1). All samples were checked for purity using the ratio of absorbance at 260 and 280, and were used for cDNA synthesis.

Table 1. Liver RNA concentrations of *Oreochromis niloticus* collected in three sampling sites.

Site	Sample No.	Concentration (ng/ul)	A280/260
Los Banos	1	1086	2.06
	2	2188.1	2.03
	3	1870.51	2.05
	4	1374.2	1.95
	5	1470.4	2.05
	6	1131.3	2.07
	7	1687.7	2.06
	8	1586.1	2.05
	9	1848.19	2.02
	10	2462.31	2
	11	1487.08	2.01
	12	1046.84	2
Sta. Rosa	1	858.4	2.01
	2	2412.86	2
	3	967.53	2.02
	4	1104.35	1.98
	5	1521.29	2.03
	6	1059.52	2.03
	7	1305.87	2.03
	8	1507.15	2.01
	9	758.36	1.93
	10	1590.57	1.95
Bohol	1	922.57	2
	2	1406.5	1.97
	3	738.23	1.97
	4	1402.94	1.99
	5	921.04	1.93
	6	1194.15	2
	7	723.37	1.96
	8	447.09	1.88
	9	553.06	1.99
	10	922.57	2

Primer Design

Primers for *O. niloticus* CYP genes and β -actin cDNA as an internal standard (Table 2) were designed using Primer Blast with melting temperatures ranging from 56 to 60 °C, and amplicon lengths of 122-429bp.

Table 2. Real-Time PCR primers of *Oreochromis niloticus* CYP and β-actin genes.

Gene name	Primer Descirption	Sequence	Amplicon Length
CYP1A	F	CCGTGACATCACAGACTCCC	429bp
	R	CACAGCTCCGGGTCATGATT	
CYP1B1	F	TTACGTCATGGCCTTCATCTAC	122bp
	R	ATGACTGTGTTCTTTGGTACGG	
CYP3A	F	CACCTCTGGGAGATTGAAAGAG	149bp
	R	TGCTGGTTACCACATCCATAC	
β-actin	F	GGGTCAGAAAGACAGCTACGTT	143bp
	R	CTCAGCTCGTTGTAGAAGGTGT	

Primer Specificity

The melting curves for each primer pair of CYP1B and CYP3A were very specific as there was an observed single temperature peak (Figure 3B and C). Meanwhile, melting curve for CYP1A (Figure 3A) shows melting curves with different peaks at different temperatures. This might be a manifestation of a higher-Tm peak and may be the result of amplicon concantenation (end-to-end annealing) in early PCR cycles. It is also possible that high concentration cDNA template was used and saturated the reaction resulting in primers binding non-specifically to other targets. It is suggested that running an agarose gel should be done to verify whether there are other, non-expected by-products.

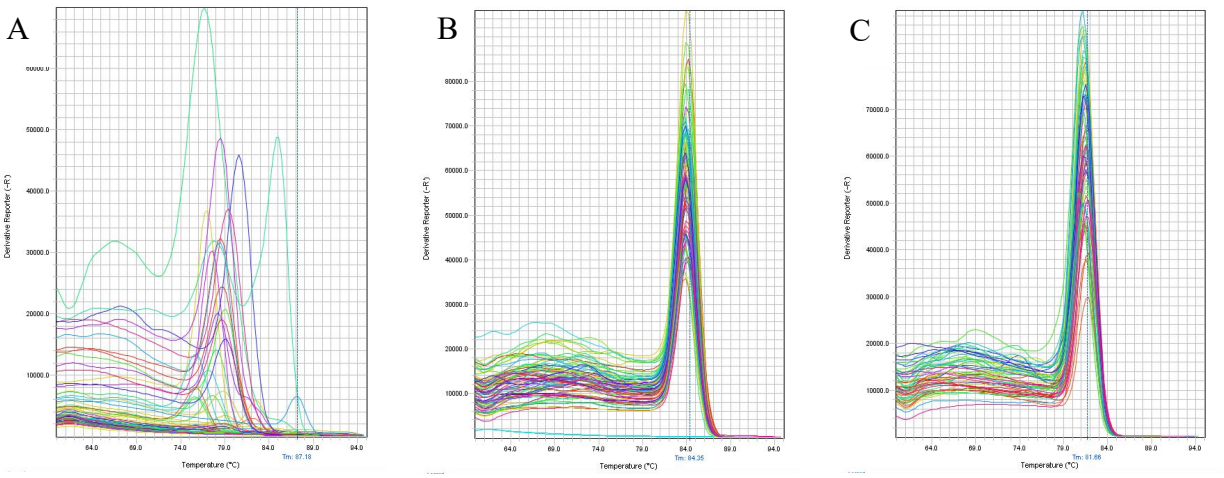

Figure 1. Melting curves of Nile tilapia CYP1A (A), CYP1B (B) and CYP3A (C) by qRT-PCR.

CYP1B mRNA expression

Results revealed that the transcript levels of cyp1b in the liver of Nile tilapia were higher in Los Banos and Bohol sites than in the Sta Rosa site (Figure 2). The expression of CYP1B1 gene is regulated by AhR, which forms an active transcription factor heterodimer with the AhR nuclear translocator (ARNT) after ligand-binding such as pesticides, and consequently induces the expression of the

CYP1B1. Observed induction of CYP1B1 in liver of Nile tilapia in the two sampling sites provided a defensive mechanism against the pollutants entering from the external environment.

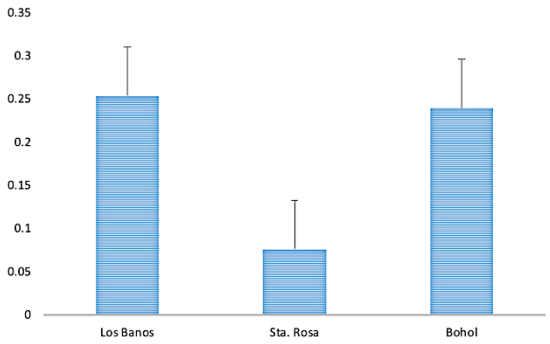

Figure 2. Nile tilapia CYP1B1 mRNA normalized to β-actin mRNA.

CYP3A mRNA expression

Similar to the *cyp1b*, the relative gene expression of *cyp3a* of Nile tilapia Los Banos and Bohol sites were higher (Figure 2). Fish from Sta Rosa expressed significantly lower mRNA. The higher concentrations of OCPs and altered levels of CYP3A in fish from Los Banos and Bohol may be associated with impacts from the agricultural activity performed in lands adjacent to the sampling sites.

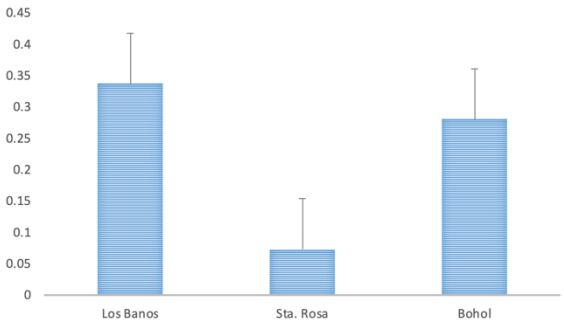

Figure 3. Nile tilapia CYP1B1 mRNA normalized to β-actin mRNA.

Agro-chemicals are one of the major sources of pollution in Laguna Lake since some of surrounding parts of the lake are agricultural areas. A study of Varca (2012) measured the concentration of pesticide residues on the surface waters of Pagsanjan and Lumban, Laguna catchment. These two rivers are major tributaries of Laguna Lake and this may load considerable amounts of pesticides since agricultural activities from both sites are active. Above the recommended level of WHO, malathion (up to 3.3 ug/L) and profenofos (up to 15.3 ug/L) were detected in Pagsanjan and Lumban, respectively.

Xenobiotic compound are being metabolized by Nile tilapia and continuous exposure to these may disrupt metabolic pathways of the fish and other organisms in the environment. Several studies suggested that Nile tilapia is the best model organism in water pollution studies because of its great sensitivity to organic pollutants. On a serious note, Nile tilapia is freely cultured in Laguna lake adjacent to Los Banos and is a common fish food source of the people living nearby. In this study,

molecular responses as predictors of fish health may serve as strong tools to observe effects not detected by classical biomarkers. Although changes in gene expression are not always directly related to a final effect, protein and gene expression and other parameters should be considered to evaluate fish health.

Perspective in Future

By far, this is among the first studies in the Philippines that revealed induction of cytochrome P450 monooxygenase genes in Nile tilapia that may be due to environmental pollutants in the Laguna de Bay. Hence, continuous monitoring of the lakes ecosystem by using the Nile tilapia as a model is highly recommended. The findings from this study can be a basis for future studies on pollutant assessment of the Philippine water system as well as measures in preserving and conserving these water systems.

Results from this study will be presented in international conferences such as SETAC World Congress in September 2020, and in national conferences such as Philippine Society for Freshwater Science in November 2020.

References:

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