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Host Sex as Factor on the Prevalence and Count of *Streptococcus* spp. in Nile Tilapia (*Oreochromis niloticus*) During the Dry and Rainy Seasons

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

The group B streptococci, particularly *Streptococcus agalactiae*, cause significant mortality and morbidity in a wide variety of freshwater and saltwater fish species in a number of countries worldwide. The possible influence of host sex in the prevalence and count of bacteria are less studied in fishes. A total of 20 Nile tilapia grow-out farms in Lubao, Pampanga, Philippines served as the collection sites during the dry and rainy seasons. The collected tilapia samples were segregated based on sex. Two series of 10-fold dilutions (10^{-1} and 10^{-2}) of tissue homogenates of tilapia organs (1 g) were separately made in Phosphate-Buffered Saline (PBS). One hundred microliters (100 µL) of the diluted organ homogenate was plated onto a selective Edwards Modified medium. The plates were incubated for 18 to 24 h at 35 to 37°C and blue to colorless colonies of *Streptococcus* spp. on plates were counted. The prevalence and count of *Streptococcus* spp. in the

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different tilapia organs based on sex and season were computed. Although not statistically significant, the results of the study showed that female tilapia were more prone to infect by *Streptococcus* spp. compared to the male fish because of higher recorded bacterial prevalence in organs such as skin, liver and brain during the dry season and in organs such as skin, liver and foregut during the rainy season. In addition, higher counts of *Streptococcus* spp. were recorded on the skin, liver and brain of female tilapia samples during both seasons.

Keywords: Aquaculture; bacteria; pond culture; immune response.

1. INTRODUCTION

The province of Pampanga in the Philippines has become a major center of fish production with its polyculture system, associating the shrimp with milkfish and sometimes tilapia and/or crabs [1]. In year 2015, tilapia production in Pampanga amounted to 109,403.35 MT and this contributed 35% to the national tilapia production of 311,684.18 MT [2].

The impact of different bacterial species on the tilapia industry is well-known among authorities because of its wide geographic distribution and the serious problems it can cause [3]. Some of the most common species of bacteria present in pond-cultured tilapia are *Streptococcus*, *Aeromonas*, *Pseudomonas*, *Vibrio*, *Staphylococcus*, *Mycobacterium*, *Edwardsiella* and *Flexibacter* [3,4].

The genus *Streptococcus* is a Gram-positive and spherical bacterium with a diameter size of less than 2 µm. It typically grows in pairs and forms chains when grown in liquid media. Most of its members are described as facultative anaerobes and catalase negative [5]. The group B streptococci, particularly *S. agalactiae*, causes significant mortality and morbidity in a wide variety of freshwater and saltwater fish species (rainbow trout, seabream, tilapia, yellowtail, catfish, croaker, killifish, menhaden, mullet and silver pomfret) in a number of countries that include United States, Israel, Japan, Kuwait, Thailand, Honduras, Costa Rica, Brazil and Colombia [6,7]. According to Pulido et al. [8], the main clinical signs observed in tilapia with streptococcosis are loss of appetite, unilateral or bilateral exophthalmia, eye hemorrhages, corneal opacity, distended abdomen, curvature of the spinal cord, stiffness, erratic swimming, and bleeding at the base of the fins. Some tilapia may have difficulty in breathing; others may not show clinical signs before death.

Most infectious diseases of fish are opportunistic, thus, other factors usually come into play in order for a disease to occur. Stressed and immunocompromised host is more susceptible to the pathogen. Some stressors that have been associated with *Streptococcus* spp. outbreaks include high water temperatures, high stocking densities, harvesting or handling, and poor water quality [9].

The possible influence of host sex in the prevalence and count of bacteria are less studied in fishes. Reimchem and Nosil [10] pointed out that there are existing evidences that males are more heavily infected than females due to the cost of sexual selection. It has been argued in some studies that competition for mates are energy costly among reproductive males, therefore, there is a possibility that males may be operating closer to their physiological limits than females [11]. Cost of reproduction for males may be especially high specially if the fish provides all the parental care and exhibits the entire territorial defense [12,13]. This can result in higher levels of stress and reduced immunocompetence in males relative to females [14]. However, differences in infection between host sexes might also arise ecologically according to Selander [15,16-17]. Meanwhile, Kennedy [18] has argued that quantitative differences in infection between sexes can be expected and may be explained as a consequence of different habitat occupied by males and females, differences in the diet and/or physiology. However, several researchers found no consistent pattern of infection in terms of prevalence and intensity between males and females [19]. This study was conducted to determine the possible influence of host sex in the prevalence and count of *Streptococcus* spp. in the various organs of Nile tilapia.

2. MATERIALS AND METHODS

2.1 Collection of Tilapia Samples

Eight hundred (800) pieces of tilapia samples reared in 20 grow-out farms in Lubao, Pampanga, Philippines from August 2017 to March 2018 were randomly collected using a cast net. In the

Philippines, dry season covers the months of December to May while the rainy season starts in June and ends by November. The four sampling periods during the rainy season were in August 12, September 10, September 28 and October 18, 2017 while the dry season samplings were conducted in January 11, February 3, February 19 and March 14, 2018.

The collected tilapia samples were stored in aerated plastic bag provided with pond water. Fish samples were transported to the Soil and Water Quality Laboratory and Fish Pathology Laboratory of the Freshwater Aquaculture Center (FAC) of the Central Luzon State University (CLSU) for analysis [20].

2.2 Segregation of Tilapia Samples Based on Sex

The collected tilapia samples were segregated which based on sex; males are characterized by the presence of pointed genital papilla with two holes, the urogenital pore and the anus, while females have rounded and pinkish genital papilla with three holes, the urinary and genital pores and the anus [20].

2.3 Quantification of *Streptococcus* spp. in the Various Organs of Tilapia Samples

The surface of the organs (skin, kidney, liver, brain and foregut) was disinfected first with 70% ethyl alcohol. Two series of 10-fold dilutions (10^{-1} and 10^{-2}) of tissue homogenates of tilapia organs (1 g) were separately made in Phosphate-Buffered Saline (PBS). One hundred microliters (100 µL) of the diluted organ was plated on Edwards Modified medium. The plates were incubated for 18 to 24 h at 35 to 37 °C. The colonies of *Streptococcus* spp. appeared bluish to colorless in the selective medium [21].

2.4 Computation of Prevalence and Count of *Streptococcus* spp.

The prevalence and count of *Streptococcus* spp. in the different tilapia organs based on sex and season was computed using the formula below [22].

% Prevalence = No. of positive samples / Tot. no. of samples x 100

CFU/mL = Average no. of colonies x dilution factor / Volume plated

CFU/mL = Summation of colony counts / [(1 x n1) + (0.1 x n2)] (d x Vp)

Where:

n_1 = no. of plates in 1st dilution counted
n_2 = no. of plates in 2nd dilution counted
d = dilution from which the 1st counts were obtained
V_p = volume plated

2.5 Statistical Analysis

Streptococcus spp. prevalence based on tilapia sex was statistically compared using independent sample T-test. *Streptococcus* counts based on sex were transformed to log10 values before subjected to independent sample T-test.

3. RESULTS

3.1 Prevalence of *Streptococcus* spp.

The prevalence of the bacterium in the various organs of the fish per farm and per season which were segregated based on sex was computed. Of the 400 samples collected during the dry season, 244 were males and 156 were females. During the dry season, the prevalence of the bacterium on the skin of the male and female tilapia ranged from 72.33 to 100.00% and 62.50 to 100.00%, respectively. In the tilapia kidney, the prevalence was 55.56 to 100.00% in males and 75.00 to 100.00% in females. The range of prevalence in the liver was 66.67 to 100.00% in males and 54.55 to 100.00% in female samples. In the brain, the prevalence of the bacterium ranged from 44.44 to 100.00% in males and 45.45 to 100.00% in females. Lastly in the foregut, prevalence ranged from 77.78 to 100.00% in males and 75.00 to 100.00% in females (Table 1).

From the 400 tilapia samples collected during the rainy season, 267 of them were identified as males and the remaining 133 samples were females. The prevalence of *Streptococcus* spp. on the skin was 50.00 to 100.00% in both male and female tilapia samples. Prevalence in the kidney ranged from 75.00 to 100.00% in males and 62.50 to 100.00% in females. In the liver, prevalence in males was 71.43 to 100% and 60.00 to 100.00% in females. The prevalence of the bacterium in the brain of male samples was 41.67 to 100.00% and 62.50 to 100.00% in females. In the foregut, the prevalence was 66.67 to 100.00% in males and 62.50 to 100.00% in females (Table 1).

Table 1. Prevalence of *Streptococcus* spp. (%) in the various organs of male and female tilapia that were collected from 20 grow-out farms in Lubao, Pampanga, Philippines during the 2018 dry and 2017 rainy seasons

	Dry		Rainy	
Organs	**Male**	**Female**	**Male**	**Female**
Skin	72.33-100.00	62.50-100.00	50.00-100.00	50.00-100.00
Kidney	55.56-100.00	75.00-100.00	75.00-100.00	62.50-100.00
Liver	66.67-100.00	54.55-100.00	71.43-100.00	60.00-100.00
Brain	44.44-100.00	45.45-100.00	41.67-100.00	62.50-100.00
Foregut	77.78-100.00	75.00-100.00	66.67-100.00	62.50-100.00

The prevalence of *Streptococcus* spp. in the various parts of male and female tilapia samples in both seasons was statistically compared in order to assess the possible influence of host sex in the bacterial presence in the population (Table 2).

The sex of tilapia did not influence the prevalence of the bacterium in the various parts of the fish during the dry season. Although this difference is not statistically significant, the results showed that higher prevalence of the bacterium was recorded from the skin (female = 93.86±10.40%, male = 93.35±8.06%), liver (female = 90.84±13.11%, male = 89.43±11.09%) and brain (female = 87.08±18.81%, male = 83.48±17.20%) of female tilapia samples; meanwhile, the bacterium was more prevalent in the kidney (male = 93.46±10.84%, female = 92.15±9.66%) and foregut (male = 98.90±2.73%, female = 95.18±8.40%) of the male samples (Table 2).

Except for the brain (male = 82.37±18.39%, female = 79.41±13.36%), the rest of the parts in females (skin = 86.45±15.68%, kidney = 93.24±10.03%, liver = 92.29±11.88%, foregut = 90.95±12.77%) had higher occurrence of the bacterium as compared to male samples (skin = 85.40±14.92%, kidney = 90.74±9.61%, liver = 92.07±8.87%, foregut = 89.76±10.22%) during the rainy season. However, these values are not statistically different, therefore, the sex of tilapia did not influence the prevalence of the bacterium in the various parts of the fish during the rainy season (Table 2).

Except for prevalence in the liver (89.43±11.09%), male tilapia samples collected during the dry season had higher prevalence of *Streptococcus* spp. on the skin (93.35±8.06%), kidney (93.46±10.84%), brain (83.48±17.20%) and foregut (98.90±2.73%) as compared to male tilapia collected during the rainy season (skin = 85.40±14.92%, kidney = 93.24±10.03%, liver = 92.07±8.87%, brain = 82.37±18.39%, foregut = 89.76±10.22%). There was significant difference on the prevalence of *Streptococcus* spp. on the skin and foregut of the male tilapia organs during the dry season (($p \leq 0.05$; Table 2).

For the female samples, the prevalence of the bacterium on the skin (93.86±10.40%), brain (87.08±18.81%) and foregut (95.18±8.40%) was higher during the dry season but not statistically significant when compared to the rainy season (skin = 86.45±15.68%, brain = 79.41±13.36%, foregut = 90.95±12.77%). In contrast, the prevalence of the bacterium in the kidney (rainy = 93.24±10.03%, dry = 92.15±9.66%) and liver (rainy = 92.29±11.88%, dry = 90.84±13.11%) of the female samples was higher during the rainy season but not statistically significant (Table 2).

Table 2. Prevalence of *Streptococcus* spp. (%) in the various organs of male and female Nile tilapia samples during the 2018 dry and 2017 rainy seasons

	Dry		Rainy	
Organs	**Male**	**Female**	**Male**	**Female**
Skin	93.35±8.06^{ax}	93.86±10.40^{ax}	85.40±14.92^{ay}	86.45±15.68^{ax}
Kidney	93.46±10.84^{ax}	92.15±9.66^{ax}	93.24±10.03^{ax}	93.24±10.03^{ax}
Liver	89.43±11.09^{ax}	90.84±13.11^{ax}	92.07±8.87^{ax}	92.29±11.88^{ax}
Brain	83.48±17.20^{ax}	87.08±18.81^{ax}	82.37±18.39^{ax}	79.41±13.36^{ax}
Foregut	98.90±2.73^{ax}	95.18±8.40^{ax}	89.76±10.22^{by}	90.95±12.77^{ax}

Male to male comparison and female to female comparison by season between columns (a-b)
Male to female comparison by season between columns (x-y)
Means (±SD) not sharing a common superscript are significantly different ($P \leq 0.05$)

3.2 Count of *Streptococcus* spp.

During the dry season, the number of the bacterium on the skin of the male and female tilapia ranged from 2.74 to 4.56 log10 CFU g^{-1} and 2.93 to 4.83 log10 CFU g^{-1}, respectively. In the kidney, the count was 2.37 to 4.91 log10 CFU g^{-1} in males and 3.12 to 4.98 log10 CFU g^{-1} in females. The bacterial population in the liver ranged from 2.35 to 4.46 log10 CFU g^{-1} in male and 1.96 to 4.66 log10 CFU g^{-1} in female samples while in the brain, the bacterial count ranged from 1.84 to 4.32 log10 CFU g^{-1} in males and 1.39 to 4.39 log10 CFU g^{-1} in females. Lastly in the foregut, bacterial count ranged from 3.26 to 4.98 log10 CFU g^{-1} in males and 2.98 to 5.01 log10 CFU g^{-1} in females (Table 3).

The farm level count of *Streptococcus* spp. on the skin of the male samples was 2.89 to 4.93 log10 CFU g^{-1} and 2.52 to 4.74 log10 CFU g^{-1} in female samples during the rainy season. Bacterial cells in the kidney were ranged from 2.89 to 4.93 log10 CFU g^{-1} in males and 2.52 to 4.74 log10 CFU g^{-1} in females. In the liver, bacterial count in males was 2.70 to 4.67 log10 CFU g^{-1} and 2.46 to 4.26 log10 CFU g^{-1} in females. The count of the bacterium in the brain of male samples was 1.24 to 4.51 log10 CFU g^{-1} and 2.27 to 5.03 log10 CFU g^{-1} in females. In the foregut, the bacterial count was 2.62 to 4.69 log10 CFU g^{-1} in males and 2.49 to 4.49 log10 CFU g^{-1} in females (Table 3).

The colony count of *Streptococcus* spp. in the various parts of male and female tilapia samples during the two seasons was statistically compared in order to determine the possible influence of host sex in the colony count of the bacterium (Table 4).

Except for foregut (male = 4.34±0.47 log10 CFU g^{-1}, female = 4.22±0.59 log10 CFU g^{-1}), results showed that female tilapia in all remaining organs (skin = 4.01±0.63 log10 CFU g^{-1}, kidney = 4.10±0.58 log10 CFU g^{-1}, liver = 3.88±0.60 log10 CFU g^{-1}, brain = 3.34±0.47 log10 CFU g^{-1}) had higher counts but not statistically significant as compared to the male tilapia (skin = 33.84±0.51 log10 CFU g^{-1}, kidney = 4.02±0.60 log10 CFU g^{-1}, liver = 3.59±0.70 log10 CFU g^{-1}, brain = 3.42±0.76 log10 CFU g^{-1}) ($P > 0.05$) (Table 4).

The comparison of bacterial counts in various parts between male and female samples during the rainy season showed non-significant differences; higher numbers were recorded on the skin (female = 3.50±0.72 log10 CFU g^{-1}, male = 3.47±0.93 log10 CFU g^{-1}), liver (female = 3.83±0.59 log10 CFU g^{-1}, male = 3.82±0.52 log10 CFU g^{-1}) and brain (female = 3.35±0.90 log10 CFU g^{-1}, male = 3.33±0.90log10 CFU g^{-1}) of female samples, meanwhile the bacterial count in male kidney (4.07±0.72 log10 CFU g^{-1}) and foregut (3.94±0.57 log10 CFU g^{-1}) was higher as compared to female counts (kidney = 3.94±0.64 log10 CFU g^{-1}, foregut = 3.79±0.66 log10 CFU g^{-1}) (Table 4).

The amount of bacterial cells on the skin (3.84±0.51 log10 CFU g^{-1}), in the brain (3.42±0.76 log10 CFU g^{-1}) and foregut (4.34±0.47 log10 CFU g^{-1}) of male tilapia samples collected during the dry season was higher as compared to those collected during the rainy season (skin = 3.47±0.93 log10 CFU g^{-1}, brain = 3.42±0.90 log10 CFU g^{-1}, foregut = 3.94±0.57 log10 CFU g^{-1}) and a statistical significant difference was detected in the number of colonies in the foregut collected in the dry season ($P \leq 0.05$). In other parts such as kidney and liver, bacterial count was higher during rainy season but not statistically significant ($P > 0.05$) (Table 4).

In females, higher bacterial count was recorded in organs such as skin, kidney, liver and foregut during the dry season; bacterial count on the skin and foregut was statistically higher during the dry season (skin = 4.01±0.63 log10 CFU g^{-1}, foregut = 4.22±0.59 log10 CFU g^{-1}) as compared to the rainy season (skin = 3.50±0.72 log10 CFU g^{-1}, foregut = 3.79±0.66 log10 CFU g^{-1}) ($P \leq 0.05$) (Table 4).

4. DISCUSSION

Interaction between the immune system and the endocrine system is well documented in fish. This interaction has been mainly studied with reference to stress hormones. Male and female sex hormones such as testosterone and estradiol-17β might also affect the various immune parameters directly during sexual maturation and spawning [23,24]. Male and female fishes commit differently in reproduction with females investing more in gamete production, while males invest more in mate attraction through the display of sexual ornamentation, which are exacerbated during the spawning period [25,26].

Table 3. Counts of *Streptococcus* spp. (CFU g^{-1}) in the various organs of male and female tilapia that were collected from 20 grow-out farms in Lubao, Pampanga, Philippines during the 2018 dry and 2017 rainy seasons

	Dry		Rainy	
Organs	**Male**	**Female**	**Male**	**Female**
Skin	2.74-4.56	2.93-4.83	2.89-4.93	2.52-4.74
Kidney	2.37-4.91	3.12-4.98	2.89-4.93	2.52-4.74
Liver	2.35-4.46	1.96-4.66	2.70-4.67	2.46-4.26
Brain	1.84-4.32	1.39-4.39	1.24-4.51	2.27-5.03
Foregut	3.26-4.98	2.98-5.01	2.62-4.69	2.49-4.49

Table 4. Counts of *Streptococcus* spp. (CFU g^{-1}) in the various organs of male and female Nile tilapia samples during the 2018 dry and 2017 rainy seasons

	Dry		Rainy	
Organs	**Male**	**Female**	**Male**	**Female**
Skin	3.90 ± 0.49^{ax}	3.94 ± 0.68^{ax}	3.47 ± 0.93^{ax}	3.50 ± 0.72^{ay}
Kidney	4.07 ± 0.63^{ax}	4.05 ± 0.61^{ax}	4.07 ± 0.72^{ax}	3.94 ± 0.64^{ax}
Liver	3.66 ± 0.71^{ax}	3.78 ± 0.69^{ax}	3.82 ± 0.52^{ax}	3.83 ± 0.59^{ax}
Brain	3.49 ± 0.73^{ax}	3.53 ± 0.85^{ax}	3.33 ± 0.90^{ax}	3.35 ± 0.90^{ax}
Foregut	4.42 ± 0.41^{ax}	4.25 ± 0.56^{ax}	3.94 ± 0.57^{ay}	3.79 ± 0.66^{ay}

Male to male comparison and female to female comparison by season between columns (a-b)
Male to female comparison by season between columns (x-y)
Means (±SD) not sharing a common superscript are significantly different ($P \leq 0.05$)

Higher prevalence of the bacterium in most parts of female tilapia can be due to the existence of an energetic trade-off between the investment in reproduction and the investment in immune responses [27], with the assumption that energy for reproduction is costly and reduces the energy for other tasks such as immunocompetence. However higher bacterial prevalence in male tilapia may be due to production of steroid hormones (mainly testosterone) that could directly affect the production of immune cells [28]. The immunosuppression by steroid hormones could result in higher bacterial level in breeding individuals or in individuals with high expression of sexual ornamentation [29,30]. In addition, the modulation of the immune system by sex steroid hormones, which influence the expression of proinflammatory and anti-inflammatory cytokines, toll-like receptors, and antibody production makes male more prone to bacterial infection. Sex hormones can impact the metabolism, growth, and virulence of pathogenic bacteria [31].

As compared to males, female tilapia samples harbor more colonies of *Streptococcus* spp. in most of their organs during both seasons. This result was also supported by the recorded higher prevalence of the bacterium in most organs of the female samples in both seasons. As stated by Sheldon and Verhulst [27], majority of the energy in female fishes are devoted to reproduction, thus, only a portion of this energy is used for immunocompetence against bacteria and other disease-causing organisms. Most research to date has concentrated on the stress responses of fish during sexual maturation. Generally, it has been found that females exhibit higher cortisol levels than males at the onset of sexual maturation, which is linked to the start of gonadal steroid production [32]. Studies in this area have particularly focused on semelparous Pacific salmon, which display hyperactivation of the HPI axis as they mature. This maturation period also corresponds with a reduction in total leukocyte numbers, lower plasma lysozyme activity in salmonid species, and a decrease in the number of antibody-producing cells [33]. Sex-based differences in immune responses have been documented in several species, with females typically displaying stronger immune reactions [34]. These responses include hypersensitivity, the release of pro-inflammatory cytokines, and antibody production. Research indicates that sex chromosomes and hormonal levels play crucial roles in these immune response differences. For instance, the G protein-coupled receptor GPR174, encoded on the X chromosome, inhibits germinal center formation in males but not in females, contributing to generally lower antibody-mediated autoimmunity in males [34]. Additionally, estrogen receptors are predominantly found in B

and T lymphocytes; higher levels of estrogen in females lead to a stronger humoral immune response [35,36].

Evidences of alterations in androgen, estrogen, and vitellogenin serum levels have been commonly documented in fish after bacterial infection [37]. For example, vibriosis outbreak in silver seabream (*Sparus sarba*) showed an increase in testosterone serum levels upon the progression of infection, while estradiol declined in moribund fish [38]. There were clear results that cortisol, growth hormone, prolactin, some proopiomelanocortin-derived peptides, and reproductive hormones from all the levels of the neuroendocrine reproductive axis regulate leucocyte functions and different immune responses in several fish species.

The study by Petersen and Davis [39] found no difference in disease susceptibility to *Edwardsiella ictaluri* infection between male and female channel catfish, although the sex of the fish might affect growth rates. However, Saha et al. [40] reported a higher prevalence of protozoan parasites in female ornamental fish compared to males, attributed to biochemical changes in the quantity and quality of steroid hormones. Similarly, Amal et al. [41] observed that the clinical signs, mortality patterns, cumulative mortality, and histopathological changes in internal organs of Javanese medaka infected with *Streptococcus agalactiae* showed no differences between all-male, all-female, and mixed-sex groups, indicating that sex does not influence susceptibility to *S. agalactiae* infection.

5. CONCLUSION

Female tilapias were more prone to infect by *Streptococcus* spp. compared to the male fish because of higher recorded bacterial prevalence in organs such as skin, liver and brain during the dry season and in organs such as skin, liver and foregut during the rainy season. In addition, higher counts of *Streptococcus* spp. were recorded on the skin, liver and brain of female tilapia samples during both seasons. This can be due to the existence of an energetic trade-off between the investment in reproduction and the investment in immune responses.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The author, Dr. Alvin T. Reyes hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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