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OXYGEN-TEMPERATURE IN THE INCIDENCE OF STREPTOCOCCUS SPP., IN FLOATING CAGES OF TILAPIA (OREOCHROMIS NILOTICUS) IN MALPASO, CHIAPAS

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— Abstract—

The present work describes the existing relationship of two environmental components in the presence of Streptococcosis in tilapia, which reports high mortality rates in open systems. For this reason, the oxygen and temperature of 10 tilapia aquaculture production units from floating cage systems and the relationship with the isolation of *Streptococcus spp*. were studied, in one of the main hydroelectric dams known as Malpaso located in Chiapas, one of the main tilapia-producing states in Mexico. Organ sampling was carried out to identify the bacteria, and at the same time oxygen and water temperature readings were taken, as well as reports of mortality and clinical signs. 80% of the units were positive for *Streptococcus spp*., With a mortality of 50% and clinical signs in 60% of the production units. The mean oxygen and temperature parameters were located at 5.5mg / l and 30.7° C, respectively. The relationship of oxygen to the incidence of *Streptococcus spp*. was found at a concentration of 5-6.5 mg / l and a temperature> 31° C. Therefore it is concluded that the environmental conditions of the floating cage systems for the production of tilapia represents a risk to the appearance of infectious diseases, due to the dynamics of the variation of biological components that puts the integrity of the fish at risk and predisposes the habitat for various pathogens such as *Streptococcus spp*.

Keywords

Mojarra; environment; mortality.

Tilapia (*Oreochromis spp.*) is one of the main species used in aquaculture production. Its ability to adapt to changing and extreme environmental conditions (Schmitter, 2006) has allowed it to be distributed in various regions of the world, at the same time, the high production efficiency and intensification of aquaculture systems have greatly favored the growth of this sector. FAO statistics show the productive increase of tilapia with annual reports ranging from 2657.7 t in 2010 to 4525.4 t in 2018, representing 8.3 % of aquaculture production worldwide. Closely matching the intensification is the demand for the product, which in *per capita* values increased from 9.0 kg (live weight) in 1961 to 20.3 kg in 2017, at an average rate of 1.5 % per year, while total meat consumption grew by 1.1 % per year in the same period (FAO, 2020).

In this sense, the intensification and expansion of aquaculture activities increase the probability of new, emerging, and re-emerging diseases (Delphino, *et al*., 2018), as well as anthropogenic activities that arise around production units, which can be associated as a cause of contamination, by physical, chemical or biological waste that alter water conditions (Soto, 2020). Therefore, it is important to differentiate the causes of disease or identify those factors that predispose to high mortality rates.

One of the most vulnerable systems is the culture of tilapia in floating cages that develops alongside the conditions of its environment, where control is practically impossible, considering currents, water level fluctuations, and quality concerning suspended substances (SADER, 2011), which compromises the adaptive capacity and survival of tilapia, due to the environmental risks that accompany it and represent a real challenge during the productive cycle (Arámbul, *et al*., 2018).

The main environmental exposures in fish are often related to spatial and temporal variation in temperature that affects physiological traits, due to their poikilothermic characteristics that compromise host resistance and to a large extent affect the colonization capacity of bacteria (Tavares, 2018).

Temperature and oxygen, together with rainy and dry periods, are considered the main climate-related risks in floating cages, as high temperatures predispose stress and susceptibility to diseases (Bahri, 2012; Lebel, 2016).

Oxygen concentrations in the water are one of the essential elements for the culture, its absence does not allow the development of tilapia, and low levels predispose a constant problem during the production cycle because at higher temperatures there is an increase in fish metabolism and higher oxygen consumption (Tomalá, *et al*., 2014).

Biological interaction also influences the incidence of facultative or opportunistic pathogens that cause mortalities ranging from 20% to 90% (García, 2020). In tilapia, *Streptococcus spp* is reported as one of the main pathogens related to water temperature and is common in intensive culture areas, especially in cage culture systems, which depend on the quality of tap

water (Kannika, 2017), this gram-positive bacterium mostly causes granulomatous infections (García, 2020), unilateral or bilateral exophthalmia, ocular opacity, hemorrhage at the base of the fins, darkening of the skin, ascites and cerebral congestion (Suanyuk, 2010) that prevent the commercialization of the product. The disease has a systemic course, reaching from 10-50% mortality in days for severely acute infections and in periods of one month, it is reported between 50-60% (Kayansamruaj, 2014; El-sayed, 2019).

In Mexico, the state of Chiapas is one of the main tilapia producers, which concentrates its production in several water bodies such as Peñitas, Malpaso, and La Angostura (Campos, 2018), however, the information available on the dynamics of various bacterial pathogens present in tilapia cultures in Chiapas is very limited. Therefore, the present study aims to identify one of the main pathogens in tilapia and its interaction with two environmental components.

MATERIALS AND METHODS

Study area

The present work was carried out in the Malpaso dam, formally called Netzahualcóyotl dam, located in the Grijalva riverbed between the municipalities of Copainalá, Tecpatán, and Ocozocoautla de Espinosa, located 2. 5 km downstream of the confluence of the La Venta and Grijalva rivers, approximately 125 km southwest of the city of Villahermosa, Tabasco, and 328 km upstream of the mouth into the Gulf of Mexico (CONAPESCA, 2015).

Isolation and identification of Streptococcus spp

The win Epi program, for aquatic organisms available online, was used to determine the minimum sample size necessary to detect disease, with a 95% confidence level of 76 production units, considering at least one infected individual, assuming a minimum expected prevalence of 0.26%, with a sample size of 10 tilapia production units in floating cage systems.

Biological material was collected from tilapia organs, including liver, spleen, brain, heart, and kidney from fattening animals weighing 250g. Dissolved oxygen (DO) and water temperature parameters were measured with the water quality tester multifunction device, and finally, mortality and presence of clinical signs were considered in each of the farms.

Sample collection

For sampling, the fish were slaughtered following NOM-033-ZOO-1995, Humane slaughter of domestic and wild animals. For this purpose, a physical

slaughter method was used, the fish were desensitized by cutting the spinal cord caudal to the skull and the upper margin of the opercula.

For the identification of *Streptococcus spp*. 25 samples were collected per production unit, corresponding to organs, which were taken by bacteriological loop and sown in an enriched medium for microorganisms, brain heart infusion (BHI), in 1.5 ml Eppendorf tubes, leaving incubation in agitation at 37 °C for 24 h, then sowing in a solid medium of 5% ram blood agar, incubating for 24 h. Colonies with *Streptococcus spp*. morphology (white colonies with beta-hemolysis halo) were identified and isolated, gram staining, CAMP, and catalase tests were performed.

Data analysis

Descriptive statistics were performed to determine the frequency and detection of *Streptococcus spp*. and correspondence analysis to identify the relationship between variables. The statistical program R version 4.0 was used.

RESULTS AND DISCUSSION

According to the evaluation of 10 aquaculture production units, an incidence of *Streptococcus spp*. of 80% was found, with 50% mortality and 60% with clinical signs. In 50% of the units, the pathogen was isolated from the liver, brain, heart, kidney, and 40% from the spleen. Table 1 shows the values found in each production unit. On average, 5.5 mg/l of oxygen and 30.7 °C of temperature were found.

Table 1
Values found in the floating cage aquaculture farms

Number of organs infected	Oxygen (mg / l)	Temperature (°C)	Mortality	Clinical signs
5	4.5	30.5	+	+
1	4	30.5	-	-
0	5	31	-	-
1	5.7	30.9	-	+
2	5	31.5	-	+
5	5.5	31.5	+	+
5	6.5	30.5	+	-
0	7	30	+	+
4	6	31	+	-
1	6	30	-	+

Source: Own elaboration

The body of water where the tilapia production units are located is one of the main hydroelectric dams that are part of the middle hydrological basin of the Grijalva, one of the most important basins in Mexico and the second most abundant in the country, with an approximate area of 58,000 km², which concentrates 42 of the 92 continental fish species (Anzueto, *et al*., 2016).

During 2015, the Management and Carrying Capacity Plan of the dam was carried out, in which some environmental parameters including temperature were evaluated. At that time a range of 23.1 °C to 28.0 °C was found, with little variation between the surface layer and a decrease at 10 and 20 m. (CONAPESCA, 2015). This data is placed below what was found (30.7° C), which suggests one of the most notable and significant modifications associated with climate change, the gradual increase in temperatures; given this, it could be necessary that in the tropics cage cultures are planned according to the region since otherwise they would no longer be feasible (Bahri, 2012).

The temperature range found (30 - 31.5° C) is in the range for *Streptococcus spp*. growth, since it only requires a temperature above 27 ° C, to activate the genetic modulation involved in its metabolism, adaptation, and pathogenicity (Tavares, 2018; Abraham, 2019), the above implies that temperature influenced the isolation of *Streptococcus spp*, in 80% of the aquaculture units.

Kayansamruaj (2014) mentions that bacterial virulence is also influenced by temperature, and in response, the tilapia exhibits massive inflammation leading to death, therefore, the higher the temperature the higher the mortality and injury rate.

Unlike temperature, oxygen was presented in a higher range, between 4 - 7 mg/l, on the other hand, this does not have such a strong effect on the bacteria, because most members of the *Streptococcus* genus are facultative anaerobes, which means that they can grow in conditions of absent or limited oxygen; they are catalase-negative with different nutritional requirements, which reflects the adaptation as a commensal (Schmitter, 2006).

Despite the success of tilapia for culture, environmental changes challenge their survival and disease resistance. Phuoc (2020) suggests that the severity and geographic range of streptococcosis outbreaks in tilapia may increase as water conditions change due to global warming, environmental pollution, and river acidification. These characteristics describe production conditions, which develop in floating cage systems, where fish are at the expense of environmental conditions. Niu (2020) found that the pathogen-positive samples corresponded entirely to cages in isolates of *Streptococcus spp*. from tilapia in floating cage and pond systems. This emphasizes the instability of this type of system, due to environmental exposure, which represents a risk for the culture and economic losses for the producer.

The unpredictability of the aquatic environment causes product losses that include production costs and biosecurity measures for the control and management of infections; on the other hand, these losses are ignored and/or not reported, due to the general acceptance of the producer who considers it within the normal in the culture, accepting margins of loss in production (Shinn, 2020).

The correlation coefficient analysis found a moderate positive correlation to the presence of *Streptococcus spp*. in temperature and oxygen, as shown in the following table.

Table 2

Correlation to the presence of Streptococcus spp

Variables	Range	*Streptococcus spp., positive*
Oxygen (mg / l)	6-6.5	0.3273
	5-5.7	0.4082
Temperature (°C)	31-31.5	0.4082

Source: Own elaboration

Assuming an effect of oxygen and temperature on the incidence of *Streptococcus spp*., we can identify that an oxygen range of 5-5.7 mg/l predisposes to the presence of the pathogen, although a range of 6-6.5 mg/l is also related, consequently we can attribute the presence of *Streptococcus spp*. to a range of 5-6.5 mg/l, since this pathogen grows in facultative anaerobic conditions, i.e., it can grow in optimal oxygen conditions or limited or even absent conditions (Schmitter, 2006). On the other hand, we observed that a temperature > 31° C predisposes to the presence of *Streptococcus spp*.

Finally, we can mention that oxygen at a concentration of 5-6.5 mg/l and a temperature > 31° C affects the incidence of *Streptococcus spp*. of tilapia cultured in floating cages. Rodkhum (2011) reports that at a temperature range of 30 - 33° C there is higher mortality due to *Streptococcus spp*. than at 25° C, on the other hand, Amal & Zamri, (2011) and Abraham (2019) mention that at a temperature > 31° C, it predisposes tilapia to outbreaks of *Streptococcus spp*. infection.

The main lesions observed in positive cases of streptococcosis in Malpaso were exophthalmia, ascites, and granulomas at the base of the tail with pus accumulations.

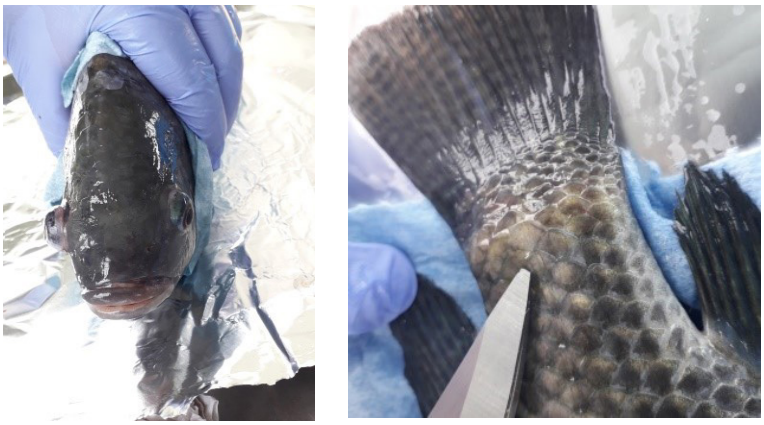

Image 1. External lesions. Unilateral exophthalmia and granuloma with purulent content

The most evident clinical signs in tilapia due to *Streptococcus spp*. from the Malpaso dam are the appearance of granulomas on the surface of the skin, on the base of the tail, which upon pressure releases a thick fluid, for this reason, it is not a product suitable for sale. Likewise, the appearance of the eye outside the orbital cavity gives an unpleasant and unhealthy appearance.

CONCLUSIONS

The average temperature found in the Malpaso dam was 30.7° C and it was found that >31°C influences the development of *Streptococcus spp*. Likewise, the oxygen level was 5.52 mg/l and at a range of 5-6.5 mg/l, it was related to the incidence of the bacteria. Thus, we can mention that the environmental conditions of floating cage systems for tilapia production represent a risk for the appearance of infectious diseases, due to the dynamics of variation that puts the integrity of the fish at risk and predisposes the habitat for diverse pathogenic and opportunistic agents.

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BIBLIOGRAPHY

Abraham, T.J., Namdeo, M.S., Adikesavalu, H., Banerjee, S. (2019). Pathogenicity and pathology of Straptococcus agalactiae in challenged Mozambique tilapia Oreochromis mossambicus (Peters 1852) juveniles. *Aquatic Research, 2*(4), 182-190. https://doi.org/10.3153/AR19017

Amal, M. & Zamri, S. M. (2011). Streptococcosis in Tilapia (Oreochromis niloticus): A Review. *Pertanika J. Trop. Agric. Sci.*, pp. 195 - 206.

Anzueto, C. M., Velázquez, V. & Gómez, G. A. E. (2016). Peces de la Reserva de la Biosfera Selva El Ocote y presa Nezahualcóyotl (Malpaso) Chiapas, México. *Revista mexicana de biodiversidad, 87*(3), pp. 972-979.

Arámbul, E. Castillo-Vargasmachuca, S., Ponce-Palafox, J., Páez-Osuna, J., Arredondo-figueroa, J., Esparza-Leal, H. (2018). Environmental variability at a marine cage culture operation in the Matanchén Bay, SE Gulf of California, Mexico. *Revista de biología marina y oceanografía, 53*(2), pp. 223-235.

Bahri, T., De Young, Y., Cochrane, K., Soto, D. (2012). *Consecuencias del cambio climático para la pesca y la acuicultura: visión de conjunto del estado actual de los conocimientos científicos*. Roma: FAO.

Campos, M. (2018). El cultivo intensivo de tilapias en jaulas flotantes: Una estrategia de seguridad alimentaria en México. En: J. Camacho, ed. *Avances de la investigación sobre producción animal y seguridad alimentaria en México*. Morelia: Universidad Michoacana de San Nicolás de Hidalgo, p. 1327.

CONAPESCA. (2015). *Ordenamiento acuícola en el Estado de Chiapas: Plan de Ordenamiento y Capacidad de carga de la presa Nezahualcóyotl (Malpaso) Primera Etapa*. México: SAGARPA.

Delphino, M., Leal, C., Gardner, I., Assis, G., Roriz, G., Ferreira, F., Figueiredo, H., Gonçalves, V. (2018). Seasonal dynamics of bacterial pathogens of Nile tilapia farmed in a Brazilian reservoir. *Aquaculture*, p. 100–108.

El -Sayed, A. (2019). *Tilapia culture*. Egipto: CABI Publishing.

FAO. (2020). *El estado mundial de la pesca y la acuicultura*. Roma, Italia: FAO.

García -Pérez, J., Ulloa, J., Mendoza-Elvira, J. (2020). Patógenos bacterianos y su resistencia a los antimicrobianos en los cultivos de tilapia en Guatemala. *Uniciencia, 35*(02), pp. 1-17.

Kannika, Pisuttharachai, D., Srisapoome, p., Wongtavatchai, J., Kondo, H., Yo hirono, Unajak, S., Areechon, N. (2017). Molecular serotyping, virulence gene profiling and pathogenicity of Streptococcus agalactiae isolated from tilapia farms in Thailand by multiplex PCR. *Journal of applied microbiology, 122*(6), pp. 1497-1507.

Kayansamruaj, P., Pirarat, N., Hirono, I., Rodkhum, Ch. (2014). Increasing of temperature induces pathogenicity of Streptococcus agalactiae and the up-regulation of inflammatory related genes in infected Nile tilapia (Oreochromis niloticus). *Veterinary microbiology*, pp. 265-271.

Lebel, L., Lebel, P., Lebel, B. (2016). Impacts, Perceptions and Management of Climate-Related Risks to Cage Aquaculture in the Reservoirs of Northern Thailand. *Environmental management, 58*(6), pp. 931-945.

Niu, G., Khattiya, R., Zhang, T., Boonyayatra, S., Wongsathein, D. (2020). Phenotypic and genotypic characterization of Streptococcus spp. isolated from tilapia (Oreochromis spp.) cultured in river-based cage and earthen ponds in Northern Thailand. *Journal of Fish Diseases, 43*(3), pp. 391-398.

Phuoc, N., Hue Linh, N., Crestani, Ch., Zadoks, R. (2020). Effect of strain and enviromental conditions on the virulence of Streptococcus agalactiae (Group B Streptococcus; GBS) in red tilapia (Oreochromis sp.). *Aquaculture*. 534

Rodkhum, Ch., Kayansamruaj, P., Pirarat, N. (2011). Effect of Water Temperature on Susceptibility to Streptococcus agalactiae Serotype Ia Infection in Nile Tilapia (Oreochromis niloticus). *The Thai Journal of Veterinary Medicine, 41*(3), p. 309.

Secretaría de Agricultura y Desarrollo Rural. (2011). *Guía empresarial para el cultivo, engorda y comercialización de la tilapia (Mojarra)*. México: SADER.

Schmitter, S. J. J., 2006. Integridad biótica y biodiversidad acuática: El caso de la tilapia africana en Quintana Roo. *Ecofronteras*, pp. 22-26.

Shinn, Andrew & Pratoomyot, Jarunan & Delannoy, Christian & Kijphakapanith, Niroj & Paladini, Giuseppe & Griffiths, Don. (2016). Calculando el costo de las enfermedades acuáticas en Asia. Panorama Acuicola Magazine. 30-33. https://www.researchgate.net/publication/304525996_Calculando_el_costo_de_las_enfermedades_acuaticas_en_Asia

Soto, D., Luque, F., Gnazzo, V. (2020). Peces de consumo humano como indicadores de contaminación ambiental por plaguicidas en el norte de Misiones, Argentina. *Rev. Argent. Salud Pública, 11*(42), pp. 7-14.

Suanyuk, N., Sukkasame, N., Tanmark, N., Yoshida, T., Itami, T., Thune, R., Tantikitti, Ch., Supamattaya, K. (2010). Streptococcus iniae infection in cultured Asian sea bass (Lates calcarifer) and red tilapia (Oreochromis sp.) in southern Thailand. *Songklanakarin Journal of Science & Technology*, pp. 341-348.

Tavares, G., Carvalho, C., Pereira, F., Rezende, C., Azevedo, V., Leal, C., Figuereido, H. (2018). Transcriptome and Proteome of Fish-Pathogenic Streptococcus agalactiae Are Modulated by Temperature. *Frontiers in Microbiology, 9*, p. 2639 .

Tomalá, D., Chavarría, J. & Escobar, B. (2014). Evaluación de la tasa de consumo de oxígeno de Colossoma macropomum en relación al peso corporal y temperatura del agua. *Latin American Journal of Aquatic Research*. *42*(5), pp. 971-979.