Influence of temperature on *Streptococcus agalactiae* infection in Nile tilapia

Influência da temperatura na infecção de tilápias do Nilo por Streptococcus agalactiae

Paulo Fernandes MARCUSSO[1]; Jefferson Yunis AGUINAGA[2]; Gustavo da Silva CLAUDIANO[1]; Silas Fernandes ETO[1]; Dayanne Carla FERNANDES[1]; Hurzana MELLO[2]; Fausto de Almeida MARINHO NETO[3]; Rogério SALVADOR[3]; Julieta Rodini Engrácia de MORAES[1]; Flávio Ruas de MORAES[1]

[1] Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Ciências Agrárias e Veterinárias, Departamento de Patologia Animal, Jaboticabal, São Paulo, Brazil
[2] Universidade Estadual Paulista "Júlio de Mesquita Filho", Centro de Aquicultura, Jaboticabal, São Paulo, Brazil
[3] Universidade do Norte do Paraná, Campus Luiz Meneghel, Bandeirantes – Paraná, Brazil

Abstract

Environmental changes affect fish homeostasis, turning them more susceptible to diseases. In Brazil, outbreaks of *Streptococcus agalactiae* infection have been reported in Nile tilapia when they are outside of their thermal comfort zone. This investigation evaluated mortality rate and which were the most infected organs at temperatures that naturally occurred in southern of Brazil. Forty Nile tilapia (*Oreochromis niloticus*) were infected with *S. agalactiae* and distributed in four groups (n=10) and each group was exposed to a different temperature: G1: 24°C, G2: 26°C, G3: 28°C, and G4: 32°C. Fish were monitored for 10 days. In this period, fish that presented irreversible clinical signs were sacrificed and samples of brain, liver, and kidney were collected for bacteriological and molecular analysis. Signs compatible with a streptococcal infection were observed in all groups. Highest mortality rates occurred at 24°C and 32°C. The brain was the most affected organ with the highest percentage of isolation of *S. agalactiae* by both methods of diagnosis. The results suggest that, as in mammals, temperatures that are further away from the comfort zone influence fish homeostasis, increasing susceptibility to bacterial infections.

Keywords: Homeostasis. *Oreochromis niloticus*. Teleost. Thermal stress.

Resumo

Mudanças ambientais afetam a homeostase dos peixes, tornando-os mais suscetíveis a doenças. No Brasil, têm sido relatados surtos de infecção por *Streptococcus agalactiae* em tilápia do Nilo, principalmente quando se encontram fora da zona de conforto térmico. No presente trabalho, foi avaliada a taxa de mortalidade e determinado quais foram os órgãos mais afetados por essa bactéria em temperaturas que ocorrem naturalmente no Sul do Brasil. Quarenta tilápias-do-nilo (*Oreochromis niloticus*) foram infectadas por *Streptococcus agalactiae* e distribuídas em quatro grupos (n = 10), cada um deles submetidos a diferentes temperaturas: G1: 24°C, G2: 26°C, G3: 28°C e G4: 32°C. Os peixes foram monitorados durante 10 dias. Os peixes com sinais clínicos irreversíveis foram sacrificados e coletadas amostras de cérebro, fígado e rins para análise bacteriológica e molecular. Foram observados sinais compatíveis com infecção estreptocócica em todos os grupos. A taxa de mortalidade mais elevada ocorreu nos grupos mantidos nas temperaturas de 24°C e 32°C. O cérebro foi o órgão mais afetado, com a maior percentagem de isolamento de *S. agalactiae* pelos dois métodos de diagnóstico. Os resultados sugerem que, tal como nos mamíferos, temperaturas que estão mais afastadas da zona de conforto afetam significativamente a homeostase dos peixes, aumentando a sua susceptibilidade para infecções bacterianas.

Palavras-chave: Homeostase. *Oreochromis niloticus*. Teleósteo. Estresse por temperatura.

Introduction

The interaction among host susceptibility, adverse environment, and pathogen defines the pathogenesis of infectious disease (SNIESKO, 1974; MARTINS et al., 2009; GULDBERG; BRUNO, 2010) and these are the largest single cause of economic losses in aquaculture.

Correspondência para:
Flávio Ruas de Moraes
Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Ciências Agrárias e Veterinárias
Via de Acesso Prof. Paulo Donato Castellane s/n
CEP 14884-900 - Jaboticabal, SP, Brazil
e-mail: fruasmoraes@gmail.com

Recebido: 22/02/2014
Aprovado: 19/01/2015

DOI: 10.11606/issn.1678-4456.v52i1p57-62

Fish depend on water to breathe, feed, excrete wastes, maintain osmolality, and reproduce. In this sense, the physical and chemical quality of an aquatic environment is critical to understand the pathogenesis of fish diseases and to develop effective preventive practices and adequate treatments (DANG; SPECK; BENKENDORFF, 2012; SOTO; REVAN, 2012).

Environmental stress leads to outbreaks of the most common diseases in fish farming (MARTINS et al., 2009). These outbreaks are associated with rapid changes in water temperature, which favors bacterial proliferation (MARCOGLIESE, 2010) and lowering host resistance (HARVELL et al., 2002; HOOPER et al., 2007).

The most commonly encountered *Streptococcus* species in fish is a group B *Streptococcus agalactiae* (MORAES; MARTINS, 2004), usually isolated from outbreaks in Brazil (SALVADOR et al., 2003, 2005; FIGUEIREDO et al., 2006) resulting in high morbidity and mortality in freshwater fish culture (KLESIUS et al., 2006).

Nile tilapia is one of the teleosts most affected by *Streptococcus* sp. (MEURER; HAYASHI; BOSCOLO, 2003) and rapid changes in temperature have a relevant role in the process (YANONG; FRANCIS-FLOYOD, 2006). Despite that the infection of *S. agalactiae* is a well known disease in tilapia, there is a lack of information about this disease in temperatures that naturally occur in southern Brazil. Our objectives were to evaluate the host susceptibility and the capability of *S. agalactiae* to infect tilapia at different temperatures, evaluating the mortality and the bacterial dissemination in the fish.

Material and Methods

Fish

Forty Nile tilapia *Oreochromis niloticus* (mean weight 40.9 ± 2.5 g) were used in this experiment. However, before of this, a subsample of the population (n = 10) was confirmed as negative for *S. agalactiae* infection by clinical and bacteriological analysis as previously described (SALVADOR et al., 2005).

Fish were maintained at 10 fish tank−1 in 80 L aquariums and fed commercial tilapia feed (28% crude protein) two times per day at 3% fish body weight. Each tank had a water flow of 1 L/min. Leftover feed and feces were siphoned weekly, and water quality parameters (pH, water temperature, dissolved oxygen, and conductivity) were measured twice a day after each feeding, using a YSI models 55 and 63 (YSI Industries, Yellow Springs, OH) (DO = 5.1 ± 1.0 mg/L; pH = 7.66 ± 0.5, and conductivity = 117.96 ± 30.5 µS/cm). These remained at comfort-zone for Nile tilapia (BOYD, 1990). Fish were acclimatized for at least one week prior to challenge and exposed to 1% non-iodinated salt bath for 60 min (FRANCIS-FLOYD et al., 1995).

Microbiologic and molecular diagnosis of Streptococcus agalactiae

Streptococcus agalactiae was isolated from naturally infected cultured Tilapia that presented signs such as dark skin, lethargy, and erratic swimming. Bacteria were identified from its culturing, morphological, tincture, and biochemical characteristics (VAN-DAMME et al., 1997; SALVADOR et al., 2005), as well as molecular analysis.

Bacterial chromosomal DNA was isolated according to the method described by Jafar et al. (2009), and molecular identification was performed as in Martinez, Harel and Gottschalk, (2001). Briefly, 16S rDNA (220-base-pair) was amplified using primers F1, 5'-GAGTTTGATCATGGCTCAG-3' and 1MOD 5'-ACCAACATGTGTTAATTACTC-3' (Genosys Biotechnologies, UK), in 50 µL reaction volume containing 25 ng of template DNA, 1.25 units of Taq DNA polymerase buffer, 0.2 pM of each primer, and 100 pMd NTPs. PCR consisted of an initial denaturation for 4 min at 94°C, followed by 35 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 55°C and extension for 1 min at 72°C. The PCR products were sequenced and compared with the sequences within the *National Center for Biotechnology Information,* NCBI (LANGE et al., 2011).

Infectivity challenge

The challenge inoculation dose was determinate based on the LC50 (test concentration that causes 50% mortality), namely 10^8 CFU/mL^{-1}, value that was previously analyzed with the Spearman- Karber software (HAMILTON; RUSSO; THURSTON, 1977). Challenge was performed by means of intracoelomic inoculation of 10^8 CFU of *Streptococcus agalactiae* in 0.5 mL of saline solution, as a single shot (SALVADOR et al., 2012).

Temperature effect

To evaluate the effect of water temperature on the clinical infection of *Streptococcus agalactiae*, four glass aquaria with 10 fish tank^{-1} were used in a completely randomized design with split-plot. Each fish represents a plot, as follows: Group 1 (G1) maintained at 24ºC; Group 2 (G2) maintained at 26ºC; Group 3 (G3) maintained at 28ºC, and group 4 (G4) maintained at 32ºC. Temperatures were altered and maintained through electric heating thermostats (Via-Aqua, US). Fish in each tank were monitored daily during the acclimation period until euthanasia.

Fish that showed irreversible clinical signs in the first 10 days were sacrificed in an alcoholic solution of benzocaine (Sigma-Aldrich Laboratory, Steinheim, Germany), at a dose of 100 mg/L^{-1} and necropsied. Immediately, samples from brain, liver, and kidney were collected, homogenized in 1× PBS, and plated in a solid medium containing BHI supplemented with 5% sheep blood, and incubated at 29°C for 7 days. After this time, all surviving fish were also sacrificed. Finally, at the end of the experiment *S. agalactiae* was determined by molecular analysis, as previously described.

Statistical analysis

A correlation between temperature and mortality was analyzed by the method of Spearman. Data obtained were subjected to a one-way Analysis of Variance (ANOVA) and Tukey test, considering the level of $p < 0.05$ (SNEDECOR; COCHRAN, 1974).

Results

There was a correlation ($p < 0.05$) between mortality and temperature after *S. agalactiae* challenge in Nile tilapia, in which 10% mortality occurred as a function of temperature. However, this correlation is low due to the groups with the highest and the lowest temperature (G1 and G4) presented both the highest mortality rates (Figure 1).

Appearance of characteristic clinical signs of an *S. agalactiae* infection occurred between the fifth and seventh day after challenge. G4 was the first to present

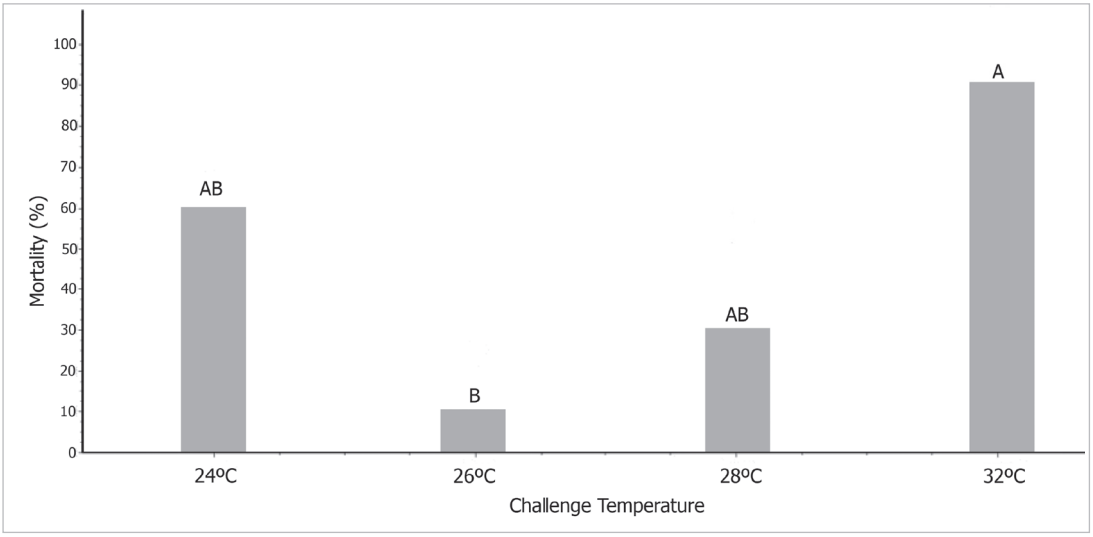

Figure 1 – Mortality in fish exposed to heat stress for 10 days. Significant differences ($p < 0.05$) are shown by dissimilar letters above bars
Source: (MARCUSSO, 2015)

signs, as lethargy, exophthalmoses, corneal opacity, erratic swimming, and cerebral congestion (Figure 2).

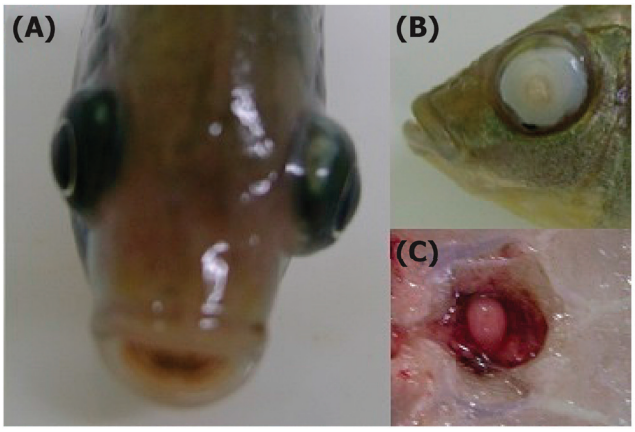

Figure 2 – (A) Exophthalmos, (B) Corneal opacity and (C) Cerebral congestion
Source: (MARCUSSO, 2015)

The bacteria was isolated in at least one of the organs collected from the 19 fish with irreversible clinical signs and the brain was the organ with the highest incidence (Table 1).

Table 1 – Results of bacterial culture in BHI of *O. niloticus* challenged with *S. agalactiae* – Brazil – 2015

Samples (n=19)	Total	Isolation	%
Brain	19	15	78,95
Liver	19	7	36,85
Kidney	19	5	26,31

Brain Heart Infusion (BHI)

Discussion

It was observed that out of the thermal comfort zone for tilapia (26 to 30°C) (BOYD, 1990; BOEGER; OSTRENSKY, 1998) fish suffer thermal stress, making them more susceptible to disease. This is probably due to a lower metabolic rate (MOURA et al., 2007) that affects cellular processes and humoral defense, mainly those that depend on metabolic energy (BALM, 1997) causing immunosuppression. It also could be explained due to reduced permeability and plasma cell migration in inflammatory response (MORAES; GARCIA LEME, 1982).

Water temperature above 27°C is a risk factor associated to outbreaks of *S. agalactiae* in Tilapia (LEAL, 2008). In this experiment, it was observed that G3 and G4 presented higher mortality rates than G2, these results which could be explained by the immunosuppression caused by release of cortisol due to the thermal stress; this affects non-specific and specific components of the immune system (FRASCÁ-SCORVO; CARNEIRO; MALHEIROS, 2001; BELO et al, 2005, 2012). Petrillo (2012) verified a decrease in the number of macrophages and granulocytes after administration of endogenous cortisol.

The development of clinical signs in the experiment was consistent with that reported in the literature (AUSTIN; AUSTIN, 2007), corroborating the results of Salvador et al. (2005) in Nile tilapia, grown in Northern Paraná, Brazil; that were similar to those found in the literature for *O. niloticus* specie and others fish affected by streptococcal infection (EVANS; KLESIUS; GILBERT, 2002; DUREMDEZ; AL-MARZOUK; QASEM, 2004).

In the group with the highest temperature (G4), some deaths occurred before manifestation of clinical signs, showing the importance of an early treatment for a satisfactory evolution of the infection. Both high and low temperatures may have interfered in host-parasite relationship. High water temperature as seen at G4 is suitable for the fast growing of *S. agalactiae* (AL-HARB, 1994; SALVADOR et al., 2005, 2012), and at the same time, being out of a fish temperature comfort zone can cause immunosuppression (BELO et al., 2005, 2012). On the other hand, a lower immune response in fish maintained in low temperature is observed probably due to a decrease of fish metabolism, becoming more susceptible to diseases.

Conclusions

The results of this study indicated that the increase fish mortality was temperature dependent. Conditions outside the fish comfort-zone determined high mortality in *Streptococcus agalactiae* infected fish, reaching nearly 100% of mortality, even before clinical signs appeared.

References

AL-HARBI, A. H. First isolation of Streptococcus sp. from hybrid tilapia (*Oreochromis niloticus x Oreochromis aureus*) in Saudi Arabia. **Aquaculture**, v. 128, p. 263-271, 1994. Available from: <http://www.sciencedirect.com/science/article/pii/0044848694903085>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1016/0044-8486(94)90308-5.

AUSTIN, B.; AUSTIN, A. D. **Bacterial fish pathogens diseases of farmed and wild fish**. 4. ed. Chichester: Praxis, 2007.

BALM, P. H. M. Immune-endocrine interactions. In: IWAMA, G. K.; PICKERING, A. D.; SUMPTER, J. P.; SCHRECK, C. B. **Fish stress and health in aquaculture**. Cambridge: Cambridge University Press, 1997. p. 195-221.

BELO, M. A. A.; MORAES, J. R. E.; SOARES, V. E.; MARTINS, M. L.; BRUM, C. D.; MORAES, F. R. Vitamin C and endogenous cortisol in foreign-body inflammatory response in pacus. **Pesquisa Agropecuária Brasileira**, v. 47, n. 7, p. 1015-1021, 2012. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-204X2012000700019>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1590/S0100-204X2012000700019.

BELO, M. A. A.; SCHALCH, S. H. C.; MORAES, F. R.; SOARES, V. E.; OTOBONI, A.; MORAES, J. E. R. Effect of dietary supplementation with vitamin E and stocking density on macrophage recruitment and giant cell formation in the teleost fish, Piaractus mesopotamicus. **Journal of Comparative Pathology**, v. 133, n. 2-3, p. 146-154, 2005. Available from: <http://www.sciencedirect.com/science/article/pii/S002199750500037X>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1016/j.jcpa.2005.04.004.

BOEGER, W.; OSTRENSKY, A. **Piscicultura**: fundamentos e técnicas de manejo. Porto Alegre: Guaíba, Agropecuária, 1998.

BOYD, C. E. **Water quality in ponds for aquaculture**. Auburn: Auburn University: Alabama Agricultural Experiment Station, 1990.

DANG, V. T.; SPECK P.; BENKENDORFF, K. Influence of elevated temperatures on the immune response of abalone, *Haliotis rubra*. **Fish & Shellfish Immunology**, v. 32, p. 732-740, 2012. Available from: <http://www.sciencedirect.com/science/article/pii/S1050464812000332>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1016/j.fsi.2012.01.022.

DUREMDEZ, R.; AL-MARZOUK, A.; QASEM, J. A. Isolation of Streptococcus agalactiae from cultured de silver pomfret, *Pampus argenteus* (Euphrasen), in Kuwait. **Journal of Fish Diseases**, v. 27, n. 5, p. 307-310, 2004. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2761.2004.00538.x/abstract>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1111/j.1365-2761.2004.00538.x.

EVANS, J. J.; KLESIUS, P. H.; GILBERT, P. M. Characterization of β-haemolytic group B *Streptococcus agalactiae* in cultured seabream, *Sparus auratus* L., and wild mullet, *Liza klunzingeri* (Day), in Kuwait. **Journal of Fish Diseases**, v. 25, n. 9, p. 505-513, 2002. Available from: <http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2761.2002.00392.x/abstract?systemMessage=Wiley+Online+Library+will+be+disrupted+on+7th+March+from+10%3A00-13%3A00+GMT+%2806%3A00-09%3A00+EST%29+for+essential+maintenance.++Apologies+for+the+inconvenience>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1046/j.1365-2761.2002.00392.x.

FIGUEIREDO, H. C. P.; CARNEIRO, D. O.; FARIA, F. C.; COSTA, G. M. Streptococcus agalactiae associado à meningoencefalite e infecção sistêmica em tilápia-do-Nilo (*Oreochromis niloticus*) no Brasil. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 58, n. 4, p. 678-680, 2006. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-09352006000400036>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1590/S0102-09352006000400036.

FRANCIS-FLOYD, R.; POYNTON, S. L.; FRASER, W.; RUTLEDGE, P.; REED, P.; NERAD, T. Spironucleus vortens from freshwater angelfish *Pterophyllum* scalare: morphology and culture. **Journal of Eukaryotic Microbiology**, v. 42, n. 6, p. 731-742, 1995. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1550-7408.1995.tb01625.x/abstract>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1111/j.1550-7408.1995.tb01625.x.

FRASCÁ-SCORVO, C. M. D.; CARNEIRO, D. J.; MALHEIROS, E. B. Comportamento alimentar do matrinxã (*Bryconcephalus*) no período de temperaturas mais baixas. **Boletim do Instituto de Pesca**, v. 27, n. 1, p. 1-5, 2001.

GULDBERG, O. H.; BRUNO, J. F. The impact of climate change on the world's marine ecosystems. **Science**, v. 328, n. 5985, p. 1523-1528, 2010. Available from: <http://www.sciencemag.org/content/328/5985/1523>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1126/science.1189930.

HAMILTON, M. A.; RUSSO, R. C.; THURSTON, R. V. Trimmed spearman-karber method for estimating median lethal concentrations in toxicity bioassays. **Environmental Science Technology**, v. 11, n. 7, p. 714-719, 1977. Available from: <http://pubs.acs.org/doi/abs/10.1021/es60130a004>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1021/es60130a004.

HARVELL, C. D.; MITCHELL, C. E.; WARD, J. R.; ALTIZER, S.; DOBSON, A. P.; OSTFELD, R. S. Climate warming and disease risks for terrestrial and marine biota. **Science**, v. 296, n. 5576, p. 2158-2162, 2002. Available from: <http://www.sciencemag.org/content/296/5576/2158>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1126/science.1063699.

HOOPER, C.; DAY, R.; SLOCOMBE, R.; HANDLINGER, J.; BENKENDORFF, K. Stress and immune responses in abalone: limitations in current knowledge and investigative methods based on other models. **Fish and Shellfish Immunology**, v. 22, n. 4, p. 363-379, 2007. Available from: <http://www.sciencedirect.com/science/article/pii/S105046480600115X>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1016/j.fsi.2006.06.009.

JAFAR, Q. A.; SAMEER, A. Z.; SALWA, A. M.; SAMEE, A. A.; AHMED, A. M.; FAISAL, A. S. Molecular investigation of *Streptococcus agalactiae* isolates from environmental samples and fish specimens during a massive fish kill in Kuwait Bay. **African Journal of Microbiology Research**, v. 1, p. 22-26, 2009. Available from: <http://www.scialert.net/abstract/?doi=pjbs.2008.2500.2504>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.3923/pjbs.2008.2500.2504.

KLESIUS, P.; EVANS, J.; SHOEMAKER, C.; YEH, H.; GOODWIN, A. E.; ADAMS, A.; THOMPSON, K. Rapid detection and identification of *Streptococcus iniae* using a monoclonal antibody-based indirect fluorescent antibody technique. **Aquaculture**, v. 258, n. 1-4, p. 180-186, 2006. Available from: <http://www.sciencedirect.com/science/article/pii/S0044848605004497>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1016/j.aquaculture.2005.06.040.

LANGE, C. C.; BRITO, M. A. V. P.; BRITO, J. R. F.; ARCURI, E. F.; SOUZA, G. N.; MACHADO, M. A.; DOMINGUES, R.; SALIMENA, A. P. S. Uso de PCR e sequenciamento do rDNA 16S para identificação de bactérias do gênero *Staphylococcus* isoladas de mastite bovina. **Pesquisa Veterinária Brasileira**, v. 1, p. 36-40, 2011. Available from: <http://www.scielo.br/scielo.php?pid=S0100-736X2011000100006&script=sci_arttext>.

Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1590/S0100-736X2011000100006.

LEAL, C. A. G. **Doenças e enfermidades na tilapicultura**.[S.l.]: Aquahelth Projetos e Consultoria, 2008.

MARCOGLIESE, D. J. The impact of climate change on the parasites and infectious diseases of aquatic animals. **OIE Revue Scientifique et Technique**, v. 27, p. 467-484, 2010.

MARTINEZ, G.; HAREL, J.; GOTTSCHALK, M. Specific detection by PCR of *Streptococcus agalactiae* in milk. **The Canadian Journal of Veterinary Research**, v. 65, n. 1, p. 68-72, 2001.

MARTINS, M. L.; MIYAZAKI, D. M. Y.; TAVARES-DIAS, M.; FENERICK, J. R. J.; ONAKA, E. M.; BOZZO, F. R.; MORAES, F. R. Characterization of the acute inflammatory response in the hybrid tambacu (*Piaractus mesopotamicus* male x *Colossoma macropomum* female) (Osteichthyes). **Brazilian Journal of Biology**, v. 69, p. 957-962, 2009. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1519-69842009000400026&lng=en&nrm=iso&tlng=en>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1590/S1519-69842009000400026.

MEURER, F.; HAYASHI, C.; BOSCOLO, W. R. Digestibilidade aparente de alguns alimentos protéicos pela tilápia do nilo (*Oreochromis niloticus*). **Revista Brasileira de Zootecnia**, v. 32, n. 6, p. 1801-1809, 2003. Available from: <http://www.scielo.br/scielo.php?pid=S1516-35982002000700001&script=sci_arttext>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1590/S1516-35982002000700001.

MORAES, F. R.; GARCIA LEME, J. Endogenous corticosteroids and insulin in acute inflammation. **Microvascular Research**, v. 23, p. 281-283, 1982. Available from: <http://www.sciencedirect.com/science/article/pii/S0026286282800010>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1016/S0026-2862(82)80001-0.

MORAES, F. R.; MARTINS, M. L. Condições predisponentes e principais enfermidades de teleósteos em piscicultura intensiva. In: CYRINO, J. E. P.; URBINATI, E. C.; CASTAGNOLLI, N. **Tópicos especiais em piscicultura de água doce tropical intensiva**. São Paulo: TecArt, 2004. v. 1. p. 343-383.

MOURA, G. S.; OLIVEIRA, M. G.; LANNA, T. A.; MACIEL-JUNIOR, A.; MACIEL, C. M. R. R. Desempenho e atividade de amilase em tilápias-do-nilo submetidas a diferentes temperaturas. **Pesquisa Agropecuária Brasileira**, v. 42, p. 1609-1615, 2007. Available from: <http://scielo.isciii.es/scielo.php?pid=S0004-05922012000300005&script=sci_arttext>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.4321/S0004-05922012000300005.

PETRILLO, T. R. **Levamisol e dexametasona na inflamação crônica por corpo estranho em Pacu (*Piaractus mesopotamicus*)**. 2012. 67 f. Dissertação. (Mestrado em Patologia Animal) – Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, 2012.

SALVADOR, R.; MÜLLER, E. E.; FREITAS, J. C.; LEONHARDT, J. H.; PRETTO-GIORDANO, L. G.; DIAS, J. A. Isolation and characterization of group B *Streptococcus* spp. from Nile tilapia (*Oreochromis niloticus*) breeding in hapas nets and in earth nurseries in the north region of Paraná State, Brazil. **Ciência Rural**, v. 35, n. 6, p. 1374-1378, 2005. Available from: <http://www.scielo.br/scielo.php?pid=S0103-84782005000600023&script=sci_arttext>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1590/S0103-84782005000600023.

SALVADOR, R.; MÜLLER, E. E.; LEONHARDT, J. H.; PRETTO-GIORDANO, L. G.; DIAS, J. A.; FREITAS, J. C.; MORENO, M. A. Isolamento de *Streptococcus* spp. de tilápias do Nilo (*Oreochromis niloticus*) e qualidade da água de tanques rede na Região Norte do Estado do Paraná, Brasil. **Semina**: ciências agrárias, v. 24, n. 1, p. 35-42, 2003. Available from: <http://www.uel.br/revistas/uel/index.php/semagrarias/article/view/2114>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.5433/1679-0359.2003v24n1p35.

SALVADOR, R.; TOAZZA, C. S.; MORAES, J. R. E.; MORAES, F. R. Inflammatory responses of Nile tilapia *Oreochromis niloticus* to *Streptococcus agalactiae*: effects of vaccination and yeast diet supplement. **Diseases of Aquatic Organisms**, v. 98, n. 3, p. 235-241, 2012. Available from: <http://www.int-res.com/abstracts/dao/v98/n3/p235-241/>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.3354/dao02438.

SNEDECOR, G. W.; COCHRAN, W. G. **Statistical methods**. Iowa: Iowa State University Press, 1974.

SNIESZKO, S. F. The effects of environmental stress on outbreaks of infectious diseases of fishes. **Journal of Fisheries Biology**, v. 6, p. 197-208, 1974. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1095-8649.1974.tb04537.x/abstract>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1111/j.1095-8649.1974.tb04537.x.

SOTO, E.; REVAN, F. Culturability and persistence of *Francisella noatunensis* subsp. orientalis (syn) in sea- and fresh water microcosms. **Microbial Ecology**, v. 63, p. 398-404, 2012. Available from: <http://link.springer.com/article/10.1007%2Fs00248-011-9932-6>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1007/s00248-011-9932-6.

VANDAMME, P.; DEVRIESE, L. A.; POT, B.; KERSTERS, K.; MELIN, P. *Streptococcus difficile* is a nonhemolytic Group B, tipe Ib *Streptococcus*. **International Journal of Systematics Bacteriology**, v. 47, p. 81-85, 1997. Available from: <http://ijs.sgmjournals.org/content/47/1/81.abstract>. Viewed: 26 Feb. 2015. doi: http://dx.doi.org/10.1099/00207713-47-1-81.

YANONG, R. P. E.; FRANCIS-FLOYD, R. **Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences. Florida**: University of Florida, 2006.