
**UNIVERSITI PUTRA MALAYSIA**

# *PREVALENCE, RISK FACTORS AND TRANSMISSION OF STREPTOCOCCUS AGALACTIAE IN THE RED HYBRID TILAPIA (OREOCHROMIS SP.)*

**MOHAMMAD NOOR AMAL BIN AZMAI**

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**By**

**MOHAMMAD NOOR AMAL BIN AZMAI**



**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy**

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**October 2011**

**Chairman: Professor Mohd Zamri Saad, DVM, PhD**
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Outbreak of *Streptococcus agalactiae* infection in fish was first reported in Malaysia in late 1990s in Pahang river, Pahang, affecting floating net cage-cultured red tilapia (*Oreochromis* sp.). Since then, outbreaks of *S. agalactiae* infection in tilapia have been widespread, covering almost all parts of Peninsular Malaysia. Presently, *S. agalactiae* infection has become a leading disease that has severe economic impact to the tilapia farming industry not only in this country but all over the world. Therefore, concrete understanding on the epidemiology of this disease is essential in order to control and prevent the infection. This study was conducted to understand the prevalence, clinical signs and pathological changes, risk factors from water quality and transmissions of *S. agalactiae* in the red hybrid tilapia (*Oreochromis* sp.) floating net cage culture system.

The prevalence of *S. agalactiae* in red hybrid tilapia cultured in different types of water bodies was investigated for a period of 24 months. The study was conducted at five types of water bodies consisting of two huge-sized reservoirs (Kenyir Lake, Terengganu and Pedu Lake, Kedah), a moderate-sized Terengganu river with four sampling sites along the river (Beladau Selat, Beladau Kepong, Pantai Ali and Kuala Kejir, Terengganu), a small-sized pond (Jitra, Kedah), a small-sized irrigation canal (Kodiang, Kedah) and a small-sized ex-mining pool (Pantai Kamloon, Penang).

This study involved monthly sampling of 30 red hybrid tilapias for bacterial isolations, measurement of water quality such as water temperature, dissolved oxygen and pH by using hand-held YSI meter (YSI Incorporated, USA) and ammonia, iron, nitrite and sulfide by using spectrophotometers (HACH Company, USA). For water flow, water clarity and depth at culture sites, the readings were measured using a current water meter (Global Water, California), Secchi disc and ultrasonic depth sensor (Speedtech Instrument, USA), respectively. All of the measurements were taken at 1 m deep involving four consistent sampling points within and surrounding the cages.

With regard to the type of water bodies, the mean prevalence of red hybrid tilapia that were cultured positive to *S. agalactiae* was significantly higher ($p<0.05$) in huge-sized with very slow water flow (0.006±0.003 cm/s) reservoirs (12.49±19.84%), compared to moderate-sized with moderate water flow (0.25±0.24 cm/s) Terengganu river (2.60±6.25%), small-sized with very slow water flow ($2.78^{-17}$±0.0 cm/s) pond (0.69±2.77%), small-sized with fast water flow (0.26±0.08 cm/s) irrigation canal (0.28±0.94%) and small-sized with very slow water flow ($2.78^{-17}$±0.00 cm/s) ex-

mining pool (0.17±0.82%). There was a significant positive correlation between the isolation of *S. agalactiae* and red hybrid tilapia mortalities ($r=0.7140$, $p<0.05$) and water temperature ($r=0.5444$, $p<0.05$) in Pedu Lake. Infections by *S. agalactiae* showed significant positive correlation to affect red hybrid tilapias of the size between 10 and 30 cm in length ($r=0.6023$, $p<0.05$). The overall mean rate of water flow from all sampling sites showed non significant negative correlation with the prevalence of red hybrid tilapia that cultured positive to *S. agalactiae* ($r=-0.2645$, $p>0.05$). There was also a significant positive correlation ($r=0.9312$, $p<0.05$) between isolations of *S. agalactiae* and mortalities of red hybrid tilapias in Pedu Lake, in the presence of *Staphylococcus* spp. The results indicate that the water temperature, rate of water flow, size of fish and the presence of other bacterial influence the prevalence of *S. agalactiae* in cultured red hybrid tilapia.

The organs of red hybrid tilapias naturally infected by *S. agalactiae* were examined for pathological changes, including histopathology. Affected red hybrid tilapias showed consistent gross findings of congestion of internal organs, particularly the livers, spleens and kidneys. Other features included exophthalmos, softening of the brains and occasional accumulation of fluid within the abdominal cavity. Microscopic examination revealed swollen endothelial cells that lined the major blood vessels of livers and occasionally spleens leading to extensive infarction, while bacterial colonies were observed within and immediately surrounding the affected blood vessels. The meninges were thickened by the infiltration of numerous heterophils. Similar infiltrations of heterophils and lymphocytes were observed in the lamina propria of intestine. The kidneys were severely congested and haemorrhagic, with

extensive interstitial nephritis. The lesion pattern suggested an acute systemic infection.

The effect of water quality on the presence of *S. agalactiae* in cultured red hybrid tilapias was only done in reservoirs and river, due to the significant isolation of the pathogen in these water bodies. There were significant differences ($p<0.05$) in water quality between lakes and river in terms of depth at culture sites, iron, ammonia, pH, sulfide, temperature, water clarity and dissolved oxygen. There were no significant differences ($p>0.05$) in iron, ammonia and nitrite levels along the river but significant differences ($p<0.05$) were recorded for the pH, water clarity, sulfide and dissolved oxygen concentration. Furthermore, the river water temperature at two upstream sites was not significantly ($p>0.05$) lower than downstream. This study revealed significant positive correlation ($r>0.5000$; $p<0.05$) between certain water quality parameters and the presence of *S. agalactiae*, particularly the pH, temperature, clarity and dissolved oxygen in both lakes. While in river, water quality parameters such as iron, ammonia, nitrite, pH, temperature, clarity and dissolved oxygen were observed to have a significant positive correlation with the presence of *S. agalactiae*. The results suggest that different type of water bodies and location of culture sites can affect water quality, creating stressed environment and increased the presence of *S. agalactiae* in cultured red hybrid tilapia.

The phenomenon on the significance of high water temperature in reservoirs and their effect to the cultured red hybrid tilapia to *S. agalactiae* infection was also investigated. Readings for water temperature and dissolved oxygen at 1 m intervals were collected *in situ* by using a hand-held YSI meter (YSI Incorporated, USA) for up

to 20 m deep, at four consistent sampling points within and surrounding the cages. In Kenyir Lake, the high water temperature column (≥29°C) was mostly noted at between 0 and 8 m depth, particularly between April and November 2007 and 2008. However, in Pedu Lake, the high water temperature (≥29°C) for up to 8 m deep was recorded between April to September 2007 and 2008. Dissolved oxygen profiling in both lakes, however, remained high at >5 mg $L^{-1}$ for up to 8 m deep, except for October 2007 in Kenyir Lake and April 2008 in Pedu Lake. Analysis of water quality parameters in Kenyir Lake revealed a significant positive correlation between water clarity ($r=0.8823$, $p<0.05$) and the prevalence of *S. agalactiae*. Significantly, water temperature also showed a negative correlation with water flow ($r=-0.8584$, $p<0.05$) and a positive correlation with water clarity ($r=0.7510$, $p<0.05$). Therefore, combinations of high water clarity, slow water flow and hot months increased the water temperature for up to 8 m deep in both reservoirs. Since red hybrid tilapias were cultured for up to ±4 m deep, this phenomenon created stressful condition and increased their susceptibility to *S. agalactiae*.

Transmission of *S. agalactiae* in red hybrid tilapias from a hatchery to a newly established farm was studied in a batch of newly hatched fry. Between 30 and 200 samples of newly hatched fry, fingerlings and adults of red hybrid tilapia, together with ten samples of water at 1 m deep and sediment were collected from a fish hatchery and farm at 15- and 30-day intervals for bacterial isolation, according to a published method in literature. Results revealed that 20% of water samples, collected on day 30 and 6.7% fingerlings from hatchery, collected on day 75 were positive to *S. agalactiae*. Following transfer of the fry to the farm, 3.3% fish that were sampled on days 180 and 210 were positive to *S. agalactiae*, while on day 270, 20% of water

samples from the farm were positive to *S. agalactiae*. Random Amplified Polymorphic DNA (RAPD-PCR) and Repetitive Polymerase Chain Reaction (REP-PCR) genotyping of the *S. agalactiae* isolates revealed no genetic diversity. This proved that the bacterial transmission was likely to occur during the fish and water transfer from the hatchery into the farm, while the bacterial probably originated from the environment of the hatchery and nearby irrigation canal.

In conclusion, the study demonstrated that combinations of water body, poor water quality, sizes of fish and the presence of other bacterial species affect the prevalence of *S. agalactiae* in the cultured red hybrid tilapia. The red hybrid tilapia that naturally infected by *S. agalactiae* also showed an acute systemic infection and the bacterial transmissions occurred during the introduction of fish and water from hatchery into the farm.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**PREVALENS, FAKTOR RISIKO DAN SEBARAN *STREPTOCOCCUS AGALACTIAE* DALAM IKAN TILAPIA MERAH HIBRID (*OREOCHROMIS* SP.)**

Oleh

**MOHAMMAD NOOR AMAL BIN AZMAI**

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Wabak jangkitan *Streptococcus agalactiae* terhadap ikan telah dilaporkan buat pertama kalinya di Malaysia pada lewat 1990an di Sungai Pahang, Pahang. Ia melibatkan jangkitan ikan tilapia merah (*Oreochromis* sp.) yang diternak di dalam sangkar jaring terapung. Sejak itu, wabak jangkitan *S. agalactiae* ke atas ikan tilapia telah merebak, meliputi sebahagian besar Semenanjung Malaysia. Kini, jangkitan *S. agalactiae* telah menjadi penyakit utama yang memberi impak ekonomi yang teruk terhadap ternakan ikan tilapia bukan saja di dalam negara, malah di seluruh dunia. Oleh itu, pemahaman menyeluruh terhadap epidemiologi penyakit ini amat penting dalam usaha untuk mengawal dan menghalang jangkitan. Kajian ini dijalankan untuk memahami prevalens, tanda klinikal dan perubahan patologi, faktor risiko dari aspek kualiti air dan sebaran jangkitan *S. agalactiae* terhadap ikan tilapia merah hibrid (*Oreochromis* sp.) yang diternak di dalam sistem sangkar jaring terapung.

Prevalens *S. agalactiae* pada ikan tilapia merah hibrid yang diternak di dalam pelbagai jenis badan air telah dikaji selama 24 bulan. Kajian telah dijalankan ke atas lima jenis badan air yang berbeza meliputi empangan yang bersaiz sangat besar (Tasik Kenyir, Terengganu dan Tasik Pedu, Kedah), Sungai Terengganu, Terengganu, yang bersaiz sederhana dan mempunyai empat tempat kajian di sepanjang sungai (Beladau Selat, Beladau Kepong, Pantai Ali and Kuala Kejir, Terengganu), kolam yang bersaiz kecil (Jitra, Kedah), terusan pengairan yang bersaiz kecil (Kodiang, Kedah) dan bekas lombong yang bersaiz kecil (Pantai Kamloon, Pulau Pinang).

Kajian melibatkan persampelan pada setiap bulan ke atas 30 ekor ikan untuk pemencilan bakteria, pengukuran kualiti air seperti suhu, oksigen terlarut dan pH dengan menggunakan meter YSI mudah alih (YSI Incorporated, USA), dan amonia, besi, nitrit dan sulfida dengan menggunakan spektrofotometer (HACH Company, USA). Pengukuran kadar pengaliran air, tahap kejernihan dan kedalaman air di setiap tempat kajian telah dilakukan dengan menggunakan meter arus (Global Water, California), disk Secchi dan sensor kedalaman ultrasonik (Speedtech Instrument, USA). Pengukuran air telah dijalankan pada tahap kedalaman 1 m pada empat tempat berbeza yang konsisten, di dalam dan kawasan yang melingkungi sangkar.

Dengan merujuk kepada jenis badan air, purata ikan tilapia merah hibrid yang dikultur positif *S. agalactiae* adalah tinggi secara signifikan ($p<0.05$) di empangan (12.49±19.84%) yang bersaiz sangar besar tetapi kadar pengaliran air yang sangat rendah (0.006±0.003 cm/s) berbanding sungai (2.60±6.25%) yang bersaiz sederhana dan kadar pengaliran air sederhana (0.25±0.24 cm/s), kolam

(0.69±2.77%) yang bersaiz kecil dan kadar aliran air yang sangat rendah ($2.78^{-17}$±0.0 cm/s), terusan pengairan (0.28±0.94%) yang bersaiz kecil dan aliran air yang laju (0.26±0.08 cm/s) dan bekas lombong (0.17±0.82%) yang bersaiz kecil dan kadar aliran air yang sangat rendah ($2.78^{-17}$±0.00 cm/s). Terdapat hubungan positif dan signifikan di antara pemencilan *S. agalactiae* dengan kematian ikan tilapia merah hibrid ($r=0.7140$, $p<0.05$) dan suhu air ($r=0.5444$, $p<0.05$) di Tasik Pedu. Jangkitan *S. agalactiae* menunjukkan hubungan signifikan dan positif ke atas ikan bersaiz di antara 10 hingga 30 cm ($r=0.6023$, $p<0.05$). Secara amnya, purata kadar aliran air dalam semua jenis badan air menunjukkan hubungan negatif tetapi tidak signifikan dengan kadar ikan tilapia merah hibrid yand dikultur positif *S. agalactiae* ($r=-0.2645$, $p>0.05$). Terdapat juga hubungan positif dan signifikan ($r=0.9312$, $p<0.05$) di antara pengasingan *S. agalactiae* dan kematian ikan tilapia merah hibrid di Tasik Pedu, dengan kehadiran *Staphylococcus* spp. Keputusan kajian menunjukkan suhu air, kadar aliran air, saiz ikan dan kehadiran bakteria lain mempengaruhi kadar prevalens *S. agalactiae* dalam ikan tilapia merah hibrid yang diternak.

Organ ikan tilapia merah hibrid yang dijangkiti *S. agalactiae* secara semulajadi telah diperiksa untuk melihat perubahan patologi, termasuk histopatologi. Ikan terjangkit menunjukkan lesi mata kasar yang konsisten, iaitu kesebakan organ dalaman, terutama hati, limpa dan buah pinggang. Penemuan lain termasuk exopthalmos, kelembutan tisu otak dan kadang-kadang pengumpulan cecair di dalam rongga badan ikan. Pemeriksaaan mikroskopik mendedahkan pembengkakan sel endothelium yang membarisi saluran darah utama hati dan kadang-kadang limpa menjurus kepada infarksi, manakala koloni bakteria dapat dilihat di dalam dan disekeliling salur darah terlibat. Selaput otak (meningis) menebal disebabkan

pengumpulan sel heterofil yang banyak. Pengumpulan sel heterofil dan limfosit yang serupa juga dilihat di dalam lamina propria usus. Ginjal menunjukkan kesebakan dan pendarahan yang teruk serta radang interstitium. Corak lesi melambangkan jangkitan sistemik yang akut.

Kesan kualiti air terhadap kehadiran *S. agalactiae* pada ikan tilapia merah hibrid hanya difokuskan kepada tasik dan sungai, disebabkan pengasingan bakteria yang signifikan daripada kedua-dua badan air tersebut. Terdapat perbezaan kualiti air yang signifikan ($p<0.05$) di antara tasik dan sungai, khususnya ke atas kedalaman tempat ternakan, tahap besi, amonia, pH, sulfida, suhu, kejernihan air dan oksigen terlarut. Perbezaan tidak signifikan ($p>0.05$) dalam air sungai melibatkan besi, amonia dan nitrit manakala perbezaan signifikan ($p<0.05$) direkodkan terhadap pH, kejernihan air, sulfida dan kepekatan oksigen terlarut. Selanjutnya, suhu air di dua tempat di hulu sungai adalah lebih rendah tetapi tidak signifikan ($p>0.05$) berbanding muara sungai. Kajian di tasik mendedahkan hubungan positif dan signifikan ($r>0.5000$; $p<0.05$) di antara beberapa parameter kualiti air dengan kehadiran *S. agalactiae.* Ini termasuk pH, suhu, kejernihan dan oksigen terlarut. Di sungai, parameter kualiti air seperti besi, amonia, nitrit, pH, suhu, kejernihan dan oksigen terlarut turut menunjukkan hubungan positif dan signifikan dengan kehadiran *S. agalactiae*. Keputusan kajian menunjukkan jenis badan air dan lokasi tempat ternakan mempengaruhi kualiti air, menyebabkan persekitaran tertekan dan meningkatkan kehadiran *S. agalactiae* dalam ikan tilapia merah hibrid yang diternak.

Fenomena suhu air yang tinggi dan signifikan di empangan dan kesannya ke atas ikan tilapia merah terjangkit *S. agalactiae* telah disiasat. Bacaan suhu air dan oksigen terlarut diambil di tempat kajian dengan menggunakan meter YSI mudah alih (YSI Incorporated, USA) sehingga kedalaman 20 m pada empat tempat berbeza yang konsisten, di dalam dan kawasan melingkungi sangkar. Di Tasik Kenyir, kolum suhu air yang tinggi (≥29$^{o}$C) dicatatkan pada kedalaman di antara 0 hingga 8 m, khususnya di antara bulan April dan November 2007 dan 2008. Walau bagaimanapun, di Tasik Pedu, suhu air yang tinggi (≥29$^{o}$C) sehingga kedalaman 8 m telah direkodkan di antara bulan April sehingga September 2007 dan 2008. Profil oksigen terlarut, bagaimana pun, kekal tinggi pada >5 mg $L^{-1}$ sehingga kedalaman 8 m, kecuali pada Oktober 2007 di Tasik Kenyir dan April 2008 di Tasik Pedu. Analisa parameter kualiti air di Tasik Kenyir menunjukkan hubungan positif dan signifikan di antara kejernihan air ($r=0.8823$, $p<0.05$) dan kadar kehadiran *S. agalactiae*. Secara signifikan, suhu air menunjukkan hubungan negatif dengan kadar aliran air ($r=-0.8584$, $p<0.05$) dan hubungan positif dengan kadar kejernihan air ($r=0.7510$, $p<0.05$). Oleh kerana itu, kombinasi kejernihan air yang tinggi, aliran air yang rendah dan bulan panas meningkatkan suhu air sehingga kedalaman 8 m di kedua-dua empangan. Memandangkan ikan tilapia merah hibrid diternak sehingga kedalaman ±4 m, fenomena ini menghasilkan keadaan tertekan dan meningkatkan kerentanan ikan terhadap *S. agalactiae*.

Sebaran *S. agalactiae* pada ikan tilapia merah hibrid dari pusat penetasan hingga ke ladang yang baru ditubuhkan telah dikaji, melibatkan satu kumpulan rega ikan tilapia merah hibrid yang baru menetas. Antara 30 sehingga 200 sampel rega yang baru menetas, anak benih dan ikan dewasa tilapia merah hibrid, bersama 10 sampel

air pada kedalaman 1 m dan mendapan telah diambil dari pusat penetasan dan ladang pada setiap selang 15 dan 30 hari untuk pemencilan bakteria, berdasarkan daripada kaedah kajian lepas. Keputusan menunjukkan 20% sampel air, yang diambil pada hari ke 30 dan 6.7% anak benih dari pusat penetasan, yang diambil pada hari ke 75 telah positif *S. agalactiae*. Selepas dipindahkan ke ladang, 3.3% ikan tilapia merah hibrid yang disampel pada hari ke 180 dan 210 telah positif *S. agalactiae*, manakala pada hari ke 270, 20% sampel air juga telah positif *S. agalactiae*. Penggunaan kaedah genotip Amplifikasi Polimorfik DNA secara Rawak (RAPD-PCR) dan Reaksi Rantai Polymerase Berulang-ulang (REP-PCR) ke atas isolat *S. agalactiae* yang dipencil dalam kajian ini menunjukkan tiada perbezaan genetik. Ini membuktikan bahawa sebaran bakteria telah berlaku semasa pemindahan ikan dan air dari pusat penetasan ke ladang ternakan, manakala bakteria ini mungkin berasal dari persekitaran pusat penetasan dan terusan pengairan yang berdekatan.

Pada kesimpulannya, kajian ini menunjukkan bahawa kombinasi badan air, tahap kualiti air yang teruk, saiz ikan dan kehadiran bakteria lain mempengaruhi prevalens *S. agalactiae* pada ikan tilapia merah hibrid yang diternak. Ikan tilapia merah hibrid yang dijangkiti *S. agalactiae* secara semulajadi menunjukkan jangkitan sistemik yang akut dan penyebaran bakteria telah berlaku semasa pemindahan ikan dan air dari pusat penetasan ke ladang.

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- My fiancée; Dr. Nurrul Shaqinah Nasrudin.

I certify that a Thesis Examination Committee has met on 3rd October of 2011 to conduct the final examination of Mohammad Noor Amal bin Azmai on his thesis entitle "Prevalence, Risk Factors and Transmission of *Streptococcus agalactiae* in the Red Hybrid Tilapia (*Oreochromis* sp.)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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## DECLARATION

I declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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**MOHAMMAD NOOR AMAL BIN AZMAI**

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# CHAPTER 1

## GENERAL INTRODUCTION

*Streptococcus* spp. are catalase negative, Gram-positive cocci bacteria with the size between 0.5 to 2.0 μm. *Streptococcus* can be isolated from fresh, brackish and marine water, while that can be either α-, β- or non-haemolytic (Evans et al., 2002). Traditional classification of Streptococci has been based on the carbohydrate antigens of the cell wall (Lancefield, 1933) and on their haemolytic activities (Evans et al., 2002). *Streptococcus* spp. had been successfully isolated from a wide range of host including human, mice, cats, dogs, hamster, camels, frogs, reptiles (Elliot et al., 1990), nutrias (Wibawan et al., 1993), monkey (Lammer et al., 1998), horses (Yildirim et al., 2002), bovines (Wibawan et al., 1991), whales (Buck et al., 1989), dolphin (Evans et al., 2006a), seals (Henton et al., 1999), porpoises (Swenshon et al., 1998) and fish (El Aamri et al., 2010).

Streptococcosis is a septicaemic disease that is caused by infection of *Streptococcus* sp. In fish, streptococcosis was initially reported in April 1957 by Hoshina et al. (1958) involving rainbow trout (*Oncorhynchus mykiss*) farmed in the Shizouka Prefecture in Japan. Later, Robinson and Meyer (1966) reported two epizootics from a private fish hatchery in United States, both involving infections of golden shiner (*Notemigonus crysoleucas)* with *Streptococcus,* while Plumb et al. (1974) successfully isolated *Streptococcus* sp. from over 50% of the diseased fish during an epizootic in estuarine bays along the Florida, Alabama and Gulf Coast of Mexico in the United States in 1972.

Since then, streptococcosis had been reported to infect a wide range of captive and wild fish in freshwater, marine and estuarine environments. The infected fish include hybrid tilapia, (*Oreochromis niloticus* x *O. aureus*) (Al-Harbi, 1994), rainbow trout, (*Oncorhynchus mykiss*) (Eldar et al., 1999a), red drum, (*Sciaenops ocellatus*) (Eldar et al., 1999b), gilthead sea bream, (*Sparus aurata*), European sea bass, (*Dicentrarchus labrax*) (Zlotkin et al., 1998), barramundi, (*Lates calcarifer*) (Bromage et al., 1999; Bromage and Owens, 2002), Japanese flounder, (*Paralichthys olivaceus*) (Nguyen et al., 2002), lined piggy, (*Pomadasys stridens*) and lizard fish, (*Synodus variegates*) (Colorni et al., 2002), seabream, (*Sparus auratus*) and wild mullet, (*Liza klunzingeri*) (Evans et al., 2002), silver pomfret, (*Pampus argenteus*) (Duremdez et al., 2004) and red hybrid tilapia, (*Oreochromis niloticus*) *(*Najiah et al., 2009; Ali et al., 2010).

Recently, streptococcal disease in tilapia has become an increasing problem and is among the leading diseases that causes severe economic impact to tilapia farming industry (Shoemaker and Klesius, 1997). The estimated economic impact of *S. agalactiae* and *S. iniae* infections in tilapia is likely to exceed USD 250 million annually (Klesius et al., 2008), while according to Evans et al. (2006b), *S. agalactiae* had been noted as one of the major tilapia pathogens among streptococcal species that affect the fish.

Reports on *S. agalactiae* infection on tilapia had been noted worldwide, including Malaysia (Salvador et al., 2005; Suanyuk et al., 2005, 2008; Garcia et al., 2010a,b; Mian et al., 2009; Najiah et al., 2009; Ali et al., 2010). In Malaysia, infection by this pathogen on tilapia has been widespread, covering almost all over Peninsular

Malaysia (Amal, 2007; Siti-Zahrah et al., 2004, 2005, 2009; Zulkafli et al., 2009; Najiah et al., 2009; Nur-Nazifah et al., 2009; Ali et al., 2010). Mortality rates were significantly higher during April to September, which was the most critical month of the year (Siti-Zahrah et al., 2009), affecting tilapias weighing between 100 and 300 g (Zulkafli et al., 2009).

There is still no comprehensive study, data or information on the epidemiology of *S. agalactiae* infection on red hybrid tilapia (*Oreochromis* sp.), which is widely cultured in various types of water bodies all over the world. In addition, the clinical signs, pathology and pathogenesis of *S. agalactiae* infection in red hybrid tilapia remained poorly understood. Furthermore, the water quality had been suspected to play a role on the susceptibility of red hybrid tilapia to streptococcus infection but was not deeply studied. Most of previous researches focused on certain water quality parameters under experimental trial, whereas the influence under true field condition with a combination of stressors from the various water quality parameters had not been studied. Similarly, the sources of *S. agalactiae* in red hybrid tilapia farm should also be deeply investigated to take an early approach to control and prevent the transmission of this pathogen.

Therefore, the objectives of this study were:

1. To determine the prevalence of *S. agalactiae* in red hybrid tilapia (*Oreochromis* sp.) kept in different types of water bodies under floating net caged culture system.
2. To determine the clinical signs and pathological changes of red hybrid tilapia (*Oreochromis* sp.) naturally infected by *S. agalactiae*.

3. To determine the effect of water quality on the presence of *S. agalactiae* in red hybrid tilapia (*Oreochromis* sp.).
4. To investigate the effect of water thermocline and dissolved oxygen profiling in lakes on the prevalence of *S. agalactiae* in red hybrid tilapia (*Oreochromis* sp.).
5. To investigate the possible sources of *S. agalactiae* in red hybrid tilapia (*Oreochromis* sp.) farms.

The hypothesis is that the water temperature, rate of water flow, size of fish and the presence of other bacterial species affect the prevalence of *S. agalactiae* in red hybrid tilapia (*Oreochromis* sp.). The red hybrid tilapias that are naturally infected by *S. agalactiae* show peculiar pathological changes slightly different from those observed under experimental infection since many water quality parameters such as water temperature, water flow, conductivity, dissolved oxygen, pH, water turbidity, un-ionized ammonia, iron, nitrite and sulfite play important role as stressor and risk factors that increase the presence of *S. agalactiae* in the cultured red hybrid tilapias, while the newly introduced fry, water and sediments from infected hatchery are the possible sources of *S. agalactiae* in red hybrid tilapia farms.

The published and submitted manuscripts in this thesis could be integrated as described below:

1. The main objectives of this study were to determine the prevalence, risk factors and transmission of *S. agalactiae* in the cage cultured red hybrid tilapia (*Oreochromis* sp.).

2. Manuscript 1: Streptococcosis in Tilapia: A Review (Most data had been published in Pertanika J. Trop. Agric. Sci., 34 (2): 195-206, 2011). This published manuscript review the current information on streptococcosis, especially on *S. agalactiae* and *S. iniae*, including their epidemiology, main water quality that contributes to the disease developments, mode of transmissions, pathogenesis, disease diagnosis and control measures in farmed tilapias. This manuscript provides information for better understanding on the main research topics of the thesis, such as tilapias and *S. agalactiae*.

3. Manuscript 2: Prevalence of *Streptococcus agalactiae* in Red Hybrid Tilapia (*Oreochromis* sp.) Kept in Different Water Bodies (Most data had been published in Online J. Vet. Res., 14 (2): 153-162, 2010). This published manuscript determined the prevalence of *S. agalactiae* in red hybrid tilapia, from nine sampling sites that consisted of five different types of water bodies, namely reservoir, river, irrigation canal, pond and ex-mining pool. Regarding on the types of water bodies, significant high prevalence of *S. agalactiae* in the cultured red hybrid tilapia was observed in reservoirs compared to the other water bodies. This study also revealed that water temperature, rate of water flow, size of fish and the presence of other bacteria influence the prevalence of *S. agalactiae* in cultured red hybrid tilapia.

4. Manuscript 3: Pathological Changes in Red Hybrid Tilapia (*Oreochromis* sp.) Naturally Infected by *Streptococcus agalactiae* (Most data had been published in J. Comp. Path., 143: 227-229, 2010). This published manuscript

documented the aspect of clinical signs and pathological changes of red hybrid tilapia that naturally infected by *S. agalactiae* of Manuscript 2. The study concluded that red hybrid tilapias, which naturally infected by *S. agalactiae* showed an acute systemic infection, compared to sub acute or chronic infection in tilapia that experimentally infected by *S. agalactiae* obtained from literatures.

5. Manuscript 4: Water Quality Influences the Presence of *Streptococcus agalactiae* in Cultured Red Hybrid Tilapia (*Oreochromis* sp.) (Submitted to Journal of Asian Fisheries Science). This submitted manuscript described the risk factors from the aspect of water quality that contributed to the presence of *S. agalactiae* in the cultured red hybrid tilapia, as observed in Manuscript 2. Due to the significant on high prevalence of *S. agalactiae* in reservoirs and river (Manuscript 2), this study was only concentrated in these two water bodies. The results showed significant positive correlations ($r>0.500$; $p<0.05$) between certain water quality parameters and the presence of *S. agalactiae*, particularly the pH, temperature, clarity and dissolved oxygen in both lakes. While in river, certain water quality parameters such as iron, ammonia, nitrite, pH, temperature, clarity and dissolved oxygen were observed to have a significant positive correlation with the presence of *S. agalactiae*. From the results, different water bodies and location of culture sites had affected the water quality, stressed the cultured fish and increased the presence of *S. agalactiae*.

6. Manuscript 5: Water Thermocline Confirms Susceptibility of Red Hybrid Tilapia (*Oeochromis* sp.) Cultured in Lakes to *Streptococcus agalactiae* (Most data had been published in J. Anim. Vet. Adv., 9 (22): 2811-2817, 2010). This published manuscript investigated on the occurrence of high water temperature and prevalence of *S. agalactiae* in reservoirs, as referred from the results of Manuscripts 2 and 4. Combinations of high water clarity, very slow rate of water flow and hot months had increased the water temperature for up to 8 m deep in reservoirs. Thus, red hybrid tilapias that were cultured at ±4 m deep were in consistent stressful culture condition and increased their susceptibility to *S. agalactiae*.

7. Manuscript 6: Hatchery as a Source of *Streptococcus agalactiae* in Red Hybrid Tilapia (*Oreochromis* sp.) Farm (Submitted to Journal of Asian Fisheries Science). This submitted manuscript discovered the possible sources of *S. agalactiae* in a newly established red hybrid tilapia farm. The results revealed that the bacterial transmission was likely to occur during the fish and water transfer from hatchery into the farm, while the bacterium probably originated from the environment of the hatchery and nearby irrigation canal. The results answered the questions on the possible sources of *S. agalactiae* infection from all of the previous Manuscripts.

## REFERENCES


Ahmed, N.A., El-Serafy, S.S., El-Shafey, A.A.M. and Abdel-Hamid, N.H. (1992). Effect of ammonia on some haematological parameters of *Oreochromis niloticus*. *Proceeding of Zoological Society of Arab Republic of Egypt* 23: 155-160.

Amal, A.M.N., Siti-Zahrah, A., Zulkafli, R., Misri, S., Ramley, A. and Zamri-Saad, M. (2008). The effect of water temperature on the incidence of *Streptococcus agalactiae* infection in cage-cultured tilapia. *International Seminar on Management Strategies on Animal Health and Production Control in Anticipation of Global Warming,* Surabaya, Indonesia. Pp. 48-51.

Baker, C.J. (1980). Group B streptococcal infection. *Advances in Internal Medicine* 25: 475-501.

Balarin, J.D. and Hatton, J.P. (1979). *Tilapia: A Guide to their Biology and Culture in Africa*. University of Stirling, Stirling, United Kingdom.

Barham, W.T., Schoobee, H. and Smit, G.L. (1979). The occurance of *Aeromonas* sp. and *Streptococcus* spp. in rainbow trout (*Salmo gairdneri*). *Journal of Fish Biology* 15: 457-460.

Baya, A.M., Lupiani, B., Hetrick, F.M., Roberson, B.S., Lukacovic, R., May, E. and Poukish, C. (1990). Association of *Streptococcus* sp. with fish mortalities in the Chespeake Bay and its tributaries. *Journal of Fish Diseases* 13: 251-253.

Berridge, B.R., Fuller, J.D., de Azavedo, J., Low, D.E., Bercovier, H. and Frelier, F. (2001). Development of a specific nested oligonucleotide PCR primer for *Streptococcus iniae* 16S-23S ribosomal DNA intergenic spacer. *Journal of Clinical Microbiology* 36: 2778-2781.

Beveridge, M.C.M. and Baird, D.J. (1998). Feeding mechanism and feeding ecology. In M.C.M. Beverigde and B.J. McAndrew (Eds.), *Tilapias: Their Biology and Exploitation* London: Chapman and Hall.

Beveridge, M.C.M. and McAndrew, B.J. (1998). *Tilapias: Their Biology and Exploitation*. London: Chapman and Hall.

Bonga, S.E.W., Flike, G. and Balm, P.H.M. (1987). Physiological adaption to acid stress in fish. *Symposium on Ecophysiology of Acid Stress in Aquatic Organism*, Brussel, Belgium. Pp. 243-254.

Boomker, J., Imes, G.D., Cameron, C.M., Naude, T. W. and Schoonbee, H. J. (1979). Trout mortalities as a result of *Streptococcus* infection. *Onderstepoort Journal of Veterinary Research* 46: 71-77.

Bowser, P.R., Wooster, G.A., Getchell, R.G. and Timmons, M.B. (1998). *Streptococcus iniae* infection of tilapia *Oreochromis niloticus* in a recirculation production facility. *Journal of World Aquaculture Society* 29: 335-339.

Boyd, E.C. and Tucker, C.S. (1998). In: *Pond Aquaculture Water Quality Management*, Kluwer Academic Publisher.

Bromage, E.S., Thomas, A. and Owens, L. (1999). *Streptococcus iniae* a bacterial infection in barramundi *Lates calcarifer*. *Diseases of Aquatic Organisms* 36: 177-181.

Bromage, E.S. and Owens, L. (2002). Infection of barramundi *Lates calcarifer* with *Streptococcus iniae*: Effect of different routes of exposure. *Diseases of Aquatic Organisms* 52: 199-205.

Buchanan, J.T., Stannard, J.A., Lauth, X., Ostland, V.E., Powell, H.C., Westerman, M.E. and Nizet, V. (2005). *Streptococcus iniae* phosphoglucomutase is a virulence factor and a target for vaccine development. *Infection and Immunity* 73: 6935-6944.

Bunch, E.C. and Bajerano, Y. (1997). The effect of environmental factors on the susceptibility of hybrid tilapia *Oreochromis niloticus* x *O. aureus* to streptococcosis. *Israeli Journal of Aquaculture* 49: 56-61.

Bullock, G.L. (1981). Streptococcal infections of fishes. *Fish Disease Leaflet*. United States Department of the Interior, Fish and Wildwife Service.

Chang, P.H. and Plumb, J.A. (1996). Histopathology of experimental *Streptococcus* sp. infection in tilapia, *Oreochromis niloticus* and channel catfish, *Ictalurus punctatus*. *Journal of Fish Diseases* 19: 235-241.

Chen, S.M., Chen, P.-C., Hsiao, I,-C. and Chen, J.-C. (2001). Effects of pH on the nitrogenous excretion and lethal DO of tilapia *Oreochromis mosasambica*. In: Sixth Asian Fisheries Forum, Book of Abstracts. Asian Fisheries Society, Manila, Philippines. Pp. 51.

Chervinski, J. (1982). Enviromental physiology of tilapia. In R.S.V. Pullin and R.H. Lowe-McConnell (Eds.), *The Biology and Culture of Tilapia*. Manila: ICLRAM. Pp. 119-128.

Cnaani, A., Gall, G.A.E. and Hulata, G. (2000). Cold tolerance of tilapia species and hybrids. *Aquaculture International* 8: 289-298.

Colorni, A., Diamant, A., Eldar, A., Kvitt, H. and Zlotkin, A. (2002). *Streptococcus iniae* infections in Red Sea cage cultured and wild fish. *Diseases of Aquatic Organisms* 49: 165-170.

Darwish, A.M. and Griffin, B.R. (2002). Study shows oxyetracycline controls *Streptococcus* in tilapia. *Global Aquaculture Advocate* 5: 34-35.

Darwish, A.M. and Hobbs, M.S. (2005). Laboratory efficacy of amoxicillin for the control of *Streptococcus iniae* infection in blue tilapia. *Journal of Aquatic Animal Health* 17: 197-202.

Duremdez, R., Al-Marzouk, A., Qasem, J.A., Al-Harbi, A. and Gharaball. H. (2004). Isolation of *Streptococcus agalactiae* from cultured silver pomfret, *Pampus argenteus,* in Kuwait. *Journal of Fish Diseases* 27: 307-310.

Eldar, A., Bejerano, Y. and Bercovier, H. (1994). *Streptococcus shiloi* and *S. difficile*, two new streptococcal species causing a meningoecephalitis in fish. *Currrent Microbiology* 28: 139-143.

Eldar, A., Bejerano, Y., Livoff, A., Horovitez, A. and Bercovier, H. (1995a). Experimental streptococcal meningo-encephalitis in cultured fish. *Veterinary Microbiology* 43: 33-40.

Eldar, A., Shaprio, O., Bejerano, Y. and Bercovier. (1995b). Vaccination with whole cell vaccine and bacterial protein extracts protects tilapia against *Streptococcus difficile* meningoencephaltis. *Vaccine* 13: 867-870.

Eldar, A., Horovitz, A. and Bercovier, H. (1997). Development of a vaccine against *Streptococcus iniae* infection in farmed rainbow trout. *Veterinary Immunology and Immunopathology* 56: 175-183.

Elliott, J.A., Facklam, R.R. and Richter, C.B. (1990). Whole-cell protein patterns ofnonhemolytic group B, type1b, streptococci isolated from humans, mice, cattle, frogs and fish. *Journal of Clinical Microbiology* 28: 628-630.

El-Sayed, A.-F.M. (2006). *Tilapia Culture*. Oceanography Department, Faculty of Science, Alexandria University, Egypt. CABI Publishing.

Evans, J.J., Klesius, P.H., Gilbert, P.M., Shoemaker, C.A., Al-Sarawi, M.A., Landsberg J., Durendez, R., Al-Marzouk, A. and Al-Zenki, S. (2002). Characterization of β-hemolytic group B *Streptococcus agalactiae* in cultured seabream, *Sparus auratus* and mullet, *Liza klunzingeri*, in Kuwait. *Journal of Fish Diseases* 25: 505-513.

Evans, J.J., Klesius, P.H. and Shoemaker, C.A. (2004). Starch hydrolysis testing of multiples isolates for rapid differentiation of *Streptococcus iniae*. *Bulletin of European Association of Fish Pathology* 24: 231-239.

Evans, J.J., Pasnik, D.J., Klesius, P.H. and Shoemaker, C.A. (2006a). Identification and epidemiology of *Streptococcus iniae* and *S. agalactiae* in tilapia, *Oreochromis* spp. International Symposium on tilapia in Aquaculture 7. Charles Town, WV, USA, American Tilapia Association. Pp. 25-42.

Evans, J.J., Pasnik, D.J., Klesius, P.H. and Al-Ablani, S. (2006b). First report of *Streptococcus agalactiae* and *Lactococcus garviae* from wild bottlenose dolphin (*Tursiops truncates*). *Journal of Wildlife Diseases* 42: 561-569.

Facklam, R., Elloitt, J., Shewmaker, L. and Reingold, A. (2005). Identification and characterization of sporadic isolates of *Streptococcus iniae* from human. *Journal of Clinical Microbiology* 43: 933-937.

FAO, (Food and Agriculture Organization of the United Nations) (2004). Fishstat Plus. FAO. Rome.

Ferguson, H., St Johns, Roach, V., Willoughby, S., Parker, C. and Ryan, R. (2000). Caribbean reef fish mortality associated with *Streptococcus iniae*. *Veterinary Record* 147: 662-664.

Fitzsimmons, K. (2000). Tilapia: the most important aquaculture species of the 21st century. In K. Fitzsimmons and J. Carvalho Filho (Eds.), *Tilapia Aquaculture in the 21st Century Fifth International Symposium on Tilapia Aquaculture*. Rio de Janeiro, Brazil. Pp. 3-8.

Fuller, J.D., Camus, A.C., Duncan, C.L., Nizet, V., Bast, D.J., Thune, Low, D.E. and de Azavedo, J.C. (2002). Identification of a streptolysin S-associated gene cluster and its role in the pathogenesis of *Streptococcus iniae* disease. *Infection and Immunity* 70: 5730-5739.

Garcia, J.C., Klesius, P.H., Evans, J.J. and Shoemaker, C.A. (2008). Non infectivity of cattle *Streptococcus agalactiae* in Nile tilapia (*Oreochromis niloticus*) and channel catfish (*Ictalurus punctatus*). *Aquaculture* 281: 151-154.

Holt, J.G., Krieg, N.R., Sheath, P.A., Staley, J.T. and William, S.T. (1994). *Bergey's Manual of Determination Bacteriology*. Philadelphia: Williams & Wilkins.

Hoshina, T., Sano, T. and Marimoto, Y. (1958). A Streptococcus pathogenic to fish. *Journal of the Tokyo University of Fisheries* 44: 57-58.

Inglis, V., Robert, R.J. and Bromage, N.R. (1993). *Bacterial Disease of Fish*. Blackwell Science Ltd, London.

Jayarao, B.M., Oliver, S.P., Matthews, K.R. and King S.H. (1991). Comparative evaluation of Vitek Gram-positive identification system and API Rapid Strep system for identification of *Streptococcus* species of bovine origin. *Veterinary Microbiology* 26: 301-308.

Kaige, N., Miyazaki, T. and Kubta, S.S. (1984). The pathogen and histophatology of vertebral deformity in cultured yellowtail. *Fish Pathology* 19: 173-179.

Kashiwagi, S., Sugimoto, N., Ohta, S. and Kusuda, R. (1977). Chemotherapeutical studies on sodium nifurstyrenate against *Streptococcus* infection in cultured yellowtail. *Fish Pathology* 12: 11-14.

Kim, J.H., Gomez, D.K., Choresca, C.H. and Park, S.C. (2007). Detection of major bacterial and viral pathogens in trash fish used to feed cultured flounder in Korea. *Aquaculture* 272: 105-110.

Kitao, T., Aoki, T. and Iwata, K. (1979). Epidemiological study on streptococcicosis of cultured yellowtail (*Seriola quinquiradiata*) 1. Distribution of *Streptococcus* sp. in sea water and muds around yellowtail farm. *Bulletin of the Japanese Society of Scientific Fisheries* 45: 567-572.

Kitao, T., Aoki, T. and Sakoh, R. (1981). Epizootic caused by β-haemolytic *Streptococcus* species in cultured freshwater fish. *Fish Pathology* 15: 301-307.

Kitao, H. (1982). Erythromycin: the application to streptococcal infection in yellowtails. *Fish Pathology* 17: 77-85.

Klesius, P.H., Shoemaker, C.A. and Evans, J.J. (1999). Efficacy of a killed *Streptococcus iniae* vaccine in tilapia (*Oreochromis niloticus*). *Bulletin of the European Association of Fish Pathologists* 19: 39-41.

Klesius, P.H., Shoemaker, C.A. and Evans, J.J. (2000). Vaccination: A health management practice for preventing diseases caused by streptococcus in tilapia and other cultured fish. In Fitzsimmons, K. and Carvalho Filho, J. (Eds.), *Tilapia Aquaculture in the 21st Century, Fifth International Symposium on Tilapia Aquaculture*. Rio de Janiero, Brazil. Pp. 558-564.

Klesius, P.H., Evans, J.J., Shoemaker, C.A and Pasnik, D.J. (2006a). Vaccines to prevent *Streptococcus iniae* and *S. agalactiae* disease in tilapia, *Oreochromis niloticus*. International symposium on tilapia in aquaculture 7. Charles Town, WV, USA, American Tilapia Association. Pp. 15-24.

Klesius, P.H., Evans, J.J., Shoemaker, C.A.,Yeh, H., Goodwin, A.E., Adams, A. and Thompson K. (2006b). Rapid detection and identification of *Streptococcus iniae* using a monoclonal antibody based indirect flourescent antibody technique. *Aquaculture* 258: 180-186.

Klesius, P.H., Shoemaker C.A. and Evans, J.J. (2008). Streptococcus: A Worldwide Fish Health Problem. *8th International Symposium on Tilapia in Aquaculture*. Cairo. Pp. 83-107.

Kvitt, K. and Colorni, A. (2004). Strain variation and geographical endemism in *Streptococcus iniae*. *Diseases of Aquatic Organisms* 61: 67-73.

Lehane, L. and Rawlin, G.T. (2000). Topically acquired bacterial zoonoses from fish: A review. *Medical Journal of Australia* 173: 256-259.

Minami, T., Nakamura, M., Ikeda, Y. and Ozaki, H. (1979). A betahemolytic *Streptococcus* isolated from cultured yellowtail. *Fish Pathology* 14: 33-38.

Nakamura, Y. (1982). Doxycycline. *Fish Pathology* 17: 67-76.

Nandlal, S. and Pickering, T. (2004). Tilapia fish farming in Pacific Island countries. *Volume 1. Tilapia Hatchery Operation*. Noumea, New Caledonia: Secretariat of the Pacific Community.

Naude, T.W. (1975). The occurrence and diagnosis of certain trout diseases. *Fish Farmer* 15: 15-20.

Nguyen, H.T., Kanai, K. and Yoshikoshi, K. (2002). Ecological investigation of *Streptococcus iniae* isolated in cultured Japanese flounder, *Paralicthys olivaceus* using selective isolation procedure. *Aquaculture* 205: 7-17.

Pasnik, D.J., Evans, J.J., Panangala. V.S., Klesius, P.H., Shelby, A. and Shoemaker, C.A. (2005). Antigenicity of *Streptococcus agalactiae* extracellular products and vaccine efficacy. *Journal of Fish Diseases* 28: 205-212.

Pier, G.B. and Madin, S.H. (1976). *Streptococcus iniae*, a beta-hemolytic *Streptococcus* isolated from Amazon freshwater dolphin, *Inia geoffrensis*. *International Journal of Systemic Bacteriology* 26: 545-553.

Pillay, T.V.R. (1990). *Aquaculture Principles and Practices*. Fishing News Books, Blackwell Science, Oxford, United Kingdom.

Plumb, J.A., Schachte, J.H., Gaines, J.L., Peltier, W. and Carrol, B. (1974). *Streptococcus* sp. from marine fishes along the Alabama and northwest Florida coast of the Gulf of Mexico. *Transactions of the American Fisheries Society* 103: 358-361.

Plumb, J.A. (1997). Infectious disease of tilapia. In: Costa-Pierce, B.A. and Rakocy, J.E. (Eds) Tilapia Aquaculture in the Americas, Vol. 1. World Aquaculture Society, Baton Rouge, Louisiana. Pp. 212-228.

Pullin, R.S.V. and Lowe-McConnel, R.H. (1982). The biology and culture of tilapias, *ICLRAM Conference Proceeding 7*, Manila: ICLRAM.

Popma, T. and Masser, M. (1999). *Tilapia Life Story and Biology*. Southern Regional Aquaculture Center Publication No. 283.

Rasheed, V. and Plumb, J.A. (1984). Pathogenicity of non-hemolytic group B *Streptococcus* sp. in gulf killfish, *Fundulus grandis*. *Aquaculture* 37: 97-105.

Roach, J.C., Levett, P.N. and Lavoie, M.C. (2006). Identification of *Streptococcus iniae* by commercial bacterial identification systems. *Journal of Microbiological Methods* 67: 20-26.

Robinson, J.A. and Meyer, F.P. (1966). Streptococcal fish pathogen. *Journal of Bacteriology* 92: 512.

Roode, M.C. (1977). *Streptococcus* sp. infection in rainbow trout. *Fish Farmer* 18: 6-8.

Salvador, R., Muller, E.E., Freitas, J.C., Leonhadt, J.H., Giordano, L.G.P. and Dias, J.A. (2005). Isolation and characterization of *Streptococcus* spp. group B in Nile tilapia (*Oreochromis niloticus*) reared in hapas nets and earth nurseries in the northen region of Parana State, Brazil. *Ciencia Rural* 35: 1374-1378.

Shelton, W.L. and Popma, T.J. (2006). *Biology: Tilapia Biology, Culture and Nutrition*. New York, USA. Food Production Press.

Shen, Z.-H., Qian, D., Xiu, W-J., Gu, J-H. and Shao, J,-Z. (2005). Isolation, identification and pathogenicity of *Streptococcus iniae* isolated from red drum *Sciaenops ocellatus*. *Acta Hydrobiologica Scadanavika* 29: 678-683.

Shiomitsu, K., Kusuda, R., Osuga, H. and Munekiyo, M. (1980). Studies on chemotherapy of fish disease with erythromycin. *Fish Pathology* 15: 17-23.

Shoemaker, C.A. and Klesius, P.H. (1997). Streptococcal disease problem and control: A Review. Tilapia Aquaculture Ithaca, NY, USA. Northwest Regional Aquaculture Engineering Service. Pp. 671-680.

Shoemaker, C.A., Klesius, P.H. and Evans, J.J. (2001). Prevalence of *Streptococcus iniae* in tilapia, hybrid striped bass and channel catfish on commercial fish farm in United States. *American Journal of Veterinary Research* 62: 174-177.

Suanyuk, N., Kanghear, H., Khongpradit, R. and Supamattaya, K. (2005). *Streptococcus agalactiae* infection in tilapia (*Oreochromis niloticus*) *Songklanakarin Journal of Science and Technology* 27: 307-319.

Sugiyama, A. and Kusuda, R. (1981). Studies on the characters of *Staphylococcus epidermidis* isolated from diseased fishes. *Fish Pathology* 16: 35-41.

Swann, L.D. (1992). A basic overview of aquaculture. *Technical Bulletin Series No.102*. Aquaculture Extension Specialist Illinois-Indiana Sea Grant Program, West Lafaette: Purdue University.

Trewaves, E. (1983). Tilapia fishes of the Genera *Sarotherodon, Oreochromis* and *Danakilia*. British Museum (Natural History).

Ugajin, M. (1981). Studies on *Streptococcus* sp. as a causal agent of an epizootic among the cultured ayu (*Plecoglossus altivelis*) in Tochigi Prefecture, Japan, 1980. *Fish Pathology* 16: 119-127.

Vandamme, L., Devriese, L.A., Kersters, P.K. and Melin, P. (1997). *Streptococcus difficile* is a non-hemolytic group B, type Ib *Streptococcus*. *International Journal of Systemic Bacteriology* 47: 81-85.

Wangead, C., Greater, A. and Tansakul, R. (1988). Effect of acid water on survival and growth rate of Nile tilapia (*Oreochromis niloticus*). In: Pullin, R.S.V., Bhukaswan, T., Tonguthai, K. and Maclean, J.L. (Eds) *Proceeding of the Second International Symposium on Tilapia in Aquaculture*. ICLRAM Conference Proceeding No. 15, Department of Fisheries, Bangkok, Thailand and ICLRAM, Manila, Philippines. Pp. 433-438.

Welcomme, R.L. (1988). International Introductions of Inland Aquatic Species, FAO Fish. Technical Paper, FAO, Rome.

Wilkinson, H.W., Thacker, L.G. and Facklam, R.R. (1973). Nonhemolytic group B streptococci of human, bovine and ichthyic origin. *Infection and Immunity* 7: 496-498.

Xu, D.H., Shoemaker, C.A. and Klesius, P.H. (2007). Evaluation of the link between grodactylosis and *Streptococcus* of Nile tilapia, *Oreochromis niloticus* (L.). *Journal of Fish Diseases* 30: 230-238.

Yanong. R.P.E. and Francis-Floyd, R. (2002). Streptococcal infections of fish. Report from University of Florida. Series from the Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Zappulli, V., Mazzariol, S., Cavicchiolo, L., Petterino, C., Bargelloni, L. and Castagnaro, M. (2005). Fatal necrotizing fasciitis and myositis in a captive common bottlenose dolphin (*Tursiops truncatus*) associated with *Streptococcus agalactiae*. *Journal of Veterinary Diagnostician and Investigation* 17: 617-622.

Zlotkin, A., Hershko, H. and Eldar, A. (1998). Possible transmission of *Streptococcus iniae* from wild fish to cultured marine fish. *Applied Environmental Microbiology* 64: 4065-4067.

sites on the presence of *S. agalactiae* in the cultured red hybrid tilapia for the better understanding of the disease epidemiology.

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## REFERENCES

Al-Harbi, A.H. and Uddin, M.N. (2004). Seasonal variation in the intestinal bacterial flora of hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) cultured in earthen ponds in Saudi Arabia. *Aquaculture* 229: 37-44.

Boyd, E.C. and Tucker, C.S. (1998). In: *Pond Aquaculture Water Quality Management*. Kluwer Academic Publisher.

Fitzsimmons, K. (2000). Tilapia: the most important aquaculture species of the 21st century. In: Tilapia Aquaculture in the 21st Century, Proceeding from the Fifth International Symposium on Tilapia Aquaculture (Eds. by Fitzsimmons, K. & Carvalho-Filho, J.). Rio de Janeiro. Pp. 3-8.

Food and Agriculture Organization, FAO (2004). World review of fisheries and aquaculture. Fisheries resources, Trends in Production, utilization and trade. Part 1. State of the world fisheries and aquaculture. FAO, Rome, Italy.

Holt, J.G., Krieg, N.R., Sheath, P.A., Staley, J.T. and William, S.T. (1994). Bergey's Manual of Determination Bacteriology (9th). Philadelphia: Williams & Wilkins.

Hoshina, T., Sano, T. and Marimoto, Y. (1958). A Streptococcus pathogenic to fish. *Journal of the Tokyo University of Fisheries* 44: 57-58.

Klesius, P.H., Shoemaker, C.A. and Evans, J.J. (2008). Streptococcus: A Worldwide Fish Health Problem. Proceedings of the 8th International Symposium on Tilapia in Aquaculture. Cairo, Egypt. Pp. 83-107.

MacMillan, J. and Santucci, T. (1990). Seasonal trends in intestinal bacterial flora of farm raised channel catfish. *Journal of Aquatic Animal Health* 2: 217-222.

Menesguen, A. and Gohin, F. (2006). Observation and modeling of natural retention structures in the English Channel. *Journal Marine Systems* 63: 244-256.

Ortega, C., Muzquiz, J.L., Docando, J., Planas, E., Alonso, J.L. and Simon, M.C. (1995). Ecopathology in aquaculture: Risk factors in infectious disease outbreaks. *Veterinary Research* 26: 57-62.

Robinson, J.A. and Meyer, F.P. (1966). Streptococcal fish pathogen. *Journal of Bacteriology* 92: 512.

Shelton, W.L. and Popma, T.J. (2006). Biology: Tilapia Biology, Culture and Nutrition. New York, USA. Food Production Press.

Siti-Zahrah, A., Padilah, B., Azila, A., Rimatulhana, R. and Shahidan, H. (2005). Multiple streptococcal species infection in cage-cultured red tilapia, but showing similar clinical sign. *Proceedings of the Sixth Symposium on Disease in Asian Aquaculture* (Eds. by Bondad-Reantaso, M.G., Mohan, C.V., Crumlish, M. and Subasinghe, R.P.). Colombo, Sri Lanka. Pp. 332-339.

Suanyuk, N., Kanghear, H., Khongpradit, R. and Supamattaya, K. (2005). *Streptococcus agalactiae* infection in tilapia (*Oreochromis niloticus*). *Songklanakarin Journal of Science and Technology* 27: 307-319.

Suresh, A.V. (1998). Tilapia update. *World Aquaculture* 30: 8-68.

Trewaves, E. (1983). Tilapine fishes of the genera *Sarotherodon, Oreochromis* and *Danakilia*. British Museum (Natural History), London.

Xu, D.H., Shoemaker, C.A. and Klesius, P.H. (2007). Evaluation of the link between gyrodactylosis and *Streptococcus* of Nile tilapia, *Oreochromis niloticus* (L.). *Journal of Fish Diseases* 30: 230-238.

## REFERENCES


Eldar, A., Bejerano, Y., Livoff, A., Horovitcz, A. and Bercovier, H. (1995). Experimental streptococcal meningo-encephalitis in cultured fish. *Veterinary Microbiology* 43: 33-40.

Evans, J.J., Wiedenmayer, A.A., Klesius, P.H. and Shoemaker, C.A. (2004). Survival of *Streptococcus agalactiae* from frozen fish following natural and experimental infections. *Aquaculture* 233: 15-21.

Evans, J.J., Klesius, P.H. and Shoemaker, C.A. (2006). An overview of streptococcus in warmwater fish. *Aquaculture Health International* 7: 10-14.

Mian, G.F., Godoy, D.T., Leal, C.A.G., Yuhara, T.Y., Costa, G.M. and Figueiredo, H.C.P. (2009). Aspects of the natural history and virulence of *Streptococcus agalactiae* infection in Nile tilapia. *Veterinary Microbiology* 136: 180-183.

Siti-Zahrah, A., Padilah, B., Azila, A., Rimatulhana, R. and Shahidan, H. (2005). Multiple streptococcal species infection in cage-cultured red tilapia, but showing similar clinical signs. *In: Proceedings of the Sixth Symposium on Diseases in Asian Aquaculture*. Eds: M.G. Bondad-Reantaso, C.V. Mohan, M. Crumlish and R.P. Subasinghe. Colombo, Sri Lanka. Pp. 332-339.

Suanyuk, N., Kong, F., Ko, D., Gilbert, G.L. and Supamattaya, K. (2008). Occurrence of rare genotypes of *Streptococcus agalactiae* in cultured red tilapia, *Oreochromis* sp. and Nile tilapia, *O. niloticus* in Thailand - Relationship to human isolates?. *Aquaculture* 284: 35-40.

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**REFERENCES**

Ali, A., Hassan, D., Saleha, A.A., Siti-Khairani, B. and Milud, A. (2010). *Streptococcus agalactiae* the etiological agent of mass mortality in farmed red tilapia (*Oreochromis* sp.) *Journal of Animal and Veterinary Advances* 9 (20): 2640-2646.

Almazan, G. and Boyd, C.E. (1978). An evaluation of Secchi disk visibility for estimating plankton densities in fish ponds. *Hydrobiologia* 65**:** 601-608.

Amal, M.N.A., Zamri-Saad, M., Siti-Zahrah, A., Zulkafli, R., Misri, S., Nur-Nazifah, M. and Shahidan, H. (2010a). Prevalence of *Streptococcus agalactiae* in tilapia kept in different water bodies. *Online Journal of Veterinary Research* 14(2): 153-162.

Amal, M.N.A., Zamri-Saad M., Zulkafli A.R., Siti-Zahrah A., Misri S., Ramley B., Shahidan H. and Sabri M.Y. (2010b). Water thermocline confirms susceptibility of tilapia cultured in lakes to *Streptococcus agalactiae*. *Journal of Animal and Veterinary Advances* 9(22): 2811-2817.

Agnew, W. and Barnes, A.C. (2007). *Streptococcus iniae*: an aquatic pathogen of global veterinary significance and a challenging candidate for reliable vaccination. *Veterinary Microbiology* 122: 1-15.

Arulampalam, P., Yusoff, F.M., Shariff, M., Law, A.T and Srinivasa Rao, P.R. (1998). Water quality and bacterial populations in a tropical marine cage culture farm. *Aquaculture Research* 29: 617-624.

Boyd, C.E. and Tucker, C.S. (1998). Pond aquaculture water quality management. Kluwer Academic Publisher.

Bunch, E.C. and Bajerano, Y. (1997). The effect of environmental factors on the susceptibility of hybrid tilapia *Oreochromis niloticus* x *O. aureus* to streptococcosis. *Israeli Journal of Aquaculture* 49: 56-61.

El-Sayed A.-F.M. (2006). Tilapia Culture. Oceanography Department, Faculty of Science, Alexandria University, Egypt. CABI Publishing.

Evans, J.J., Shoemaker, C.A. and Klesius, P.H. (2003). Effects of sublethal dissolved oxygen stress on blood glucose and susceptibility to *Streptococcus agalactiae* in Nile tilapia, *Oreochromis niloticus*. *Journal of Aquatic Animal Health* 15: 202-208.

Evans, J.J., Pasnik, D.J., Bril, G.C. and Klesius P.H. (2006). Un-ionized ammonia exposure in Nile tilapia: Toxicity, stress response and susceptibility to *Streptococcus agalactiae*. *North American Journal of Aquaculture* 68(1): 23-33.

Gonzalez, R.J., Grippo, R.S. and Dunson, W.A. (1990). The disruption of sodium balance in brook charr, *Salvelinus jbntinalis* (Mitchill), by manganese and iron. *Journal of Fish Biology* 37: 765-774.

Hansen, G.H. and Olafsen, J.A. (1999). Bacterial interactions in early life stages of marine cold water fish. *Microbial Ecology* 38: 1-26.

Heckman, C.W., dos Campos, J.L.E. and Hardoim, E.L. (1997). Nitrite concentration in well water from Poconé, Mato Grosso, and its relationship to public health in rural Brazil. *Bulletin Environmental Contamination and Toxicology* 58: 8-15.

Holt, J.G., Krieg, N.R., Sheath, P.A., Staley, J.T. and William, S.T. (1994). Bergey's Manual of Determination Bacteriology (9th). Philadelphia: Williams & Wilkins.

Hoshina, T., Sano, T. and Marimoto, Y. (1958). A Streptococcus pathogenic to fish. *Journal of the Tokyo University of Fisheries* 44: 57-58.

Hurvitz, A., Bercovier, H. and Van Rijn, J. (1997). Effect of ammonia on the survival and the immune response of rainbow trout (*Oncorhynchus mykiss*, Walbaum) vaccinated against *Streptococcus iniae*. *Fish and Shellfish Immunology* 7: 45-53.

Menesguen, A. and Gohin, F. ( 2006). Observation and modeling of natural retention structures in the English Channel. *Journal of Marine Systems* 63: 244-256.

Mian, G.F., Godoy, D.T., Leal, C.A.G., Yuhara, T.Y., Costa, G.M. and Figueiredo, H.C.P., (2009). Aspects of the natural history and virulence of *Streptococcus agalactiae* infection in Nile tilapia. *Veterinary Microbiology* 136: 180-183.

Muzquiz, J.L., Royo, F.M., Ortega, C., De Blias, I., Ruiz, I. and Alonso, J.L. (1999). Pathogenicity of streptococcosis in rainbow trout (*Oncorhynchus mykiss)* dependence on age of diseased fish. *Bulletin of the European Association of Fish Pathologists* 19: 114-119.

Najiah, M., Lee, S.W., Nadirah, M., Ruhil, H., Lee, K.L., Wendy, W., Amal, M.N.A., Basiriah, M.K. and Siti-Zahrah, A. (2009). Streptococcosis in red hybrid tilapia (*Oreochromis niloticus*) commercial farms in Malaysia. *Aquaculture Research* 40: 630-632.

Nguyen, H.T., Kanai, K. and Yoshikoshi, K. (2002). Ecological investigation of *Streptococcus iniae* isolated in cultured Japanese flounder, *Paralicthys olivaceus* using selective isolation procedure. *Aquaculture* 205: 7-17.

Ortega, C., Muzquiz, J.L., Fernandez, A., Ruiz, I., De Blas, I., Simon, M.C. and Alonso, J.L. (1996). Water quality parameters associated with *Aeromonas* spp.-affected hatcheries. *Veterinary Research* 27: 553-560.

Perera, R.P., Sterling, K.J. and Lewis, D.H. (1997). Epizootiological aspects of *Streptococcus iniae* affecting tilapia in Texas. *Aquaculture* 152: 25-33.

Randall, D.J. and Tsui, T.K.N. (2002). Ammonia toxicity in fish. *Marine Pollution Bulletin* 45: 17-23.

Robinson, J.A. and Meyer, F.P. (1966). Streptococcal fish pathogen. *Journal of Bacteriology* 92: 512.

Shoemaker, C. and Klesius, P. (1997). Streptococcal disease problems and control: a review. In: Fitzsimmons, K. (Ed). Tilapia Aquaculture Vol. 2 Northeast Regional Agricultural Engineering Service-106, Ithaca, NY, USA, Pp. 671-680.

Siti-Zahrah A., Padilah, B., Azila, A., Rimatulhana, R. and Shahidan, H. (2005). Multiple streptococcal species infection in cage-cultured red tilapia, but showing similar clinical sign. Eds: Bondad-Reantaso, M.G., C.V., Mohan, M., Crumlish, and R.P. Subasinghe. *Proceedings of the Sixth Symposium on Disease in Asian Aquaculture*. Colombo, Sri Lanka. Pp. 332-339.

Suanyuk, N., Kong, F., Ko, D., Gwendolyn, L.G. and Supamattaya, K. (2008). Occurrence of rare genotypes of *Streptococcus agalactiae* in cultured red tilapia *Oreochromis* sp. and Nile tilapia *O. niloticus* in Thailand-Relationship to human isolates?. *Aquaculture* 284: 35-40.

Verschuere, L., Rombaut, G., Sorgeloos, P. and Verstraete, W. (2000). Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Reviews* 64: 655-671.

Winton, J.R. (2001). Fish health management, In: Wedemeyer, G. (Ed.), Fish Hatchery Management, 2nd Edition. American Fisheries Society, Bethesda, MD. Pp. 559-639.

Zamri-Saad, M., Amal, M.N.A. and Siti-Zahrah, A. (2010). Pathological changes in red tilapias (*Oreochromis* spp.) naturally infected by *Streptococcus agalactiae*. *Journal of Comparative Pathology* 143: 227-229.

present study, the DO was not as low as expected probably due to the high water clarity that enhanced deeper light penetration and photosynthesis process.

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## REFERENCES

Almazan, G. and Boyd, C.E. (1978). An evaluation of Secchi disk visibility for estimating plankton densities in fish ponds. *Hydrobiologia* 65: 601-608.

Amal, M.N.A., Zamri-Saad, M., Siti-Zahrah, A., Zulkafli, R., Misri, S., Nur-Nazifah, M. and Shahidan H. (2010). Prevalence of *Streptococcus agalactiae* in tilapia kept in different water bodies. *Online Journal of Veterinary Research* 11(2): 153-162.

Bowser, P.R., Wooster, G.A., Getchell, R.G. and Timmons, M.B. (1998). *Streptococcus iniae* infection of tilapia *Oreochromis niloticus* in a recirculation production facility. *Journal of World Aquaculture Society* 29: 335-339.

Boyd, E.C. and Tucker, C.S. (1998). In: *Pond Aquaculture Water Quality Management*. Kluwer Academic Publisher.

Bunch, E.C. and Bajerano, Y. (1997). The effect of environmental factors on the susceptibility of hybrid tilapia *Oreochromis niloticus* x *O. aureus* to streptococcosis. *Israeli Journal of Aquaculture* 49: 56-61.

Chang, P.H. and Plumb, J.A. (1996). Histopathology of experimental *Streptococcus* sp. infection in tilapia, *Oreochromis niloticus* (L.) and channel catfish, *Ictalurus punctatus* (Rafinesque). *Journal of Fish Diseases* 19: 235-241.

El-Sayed, A.-F.M., El-Ghobashy, A. and Al-Amoudi, M.M. (1996). Effects of pond depth and water temperature on the growth, mortality and body composition of Nile tilapia, *Oreochromis niloticus* (L). *Aquaculture Research* 27: 681-687.

Evans, J.J., Klesius, P.H., Gilbert, P.M., Shoemaker, C.A., Al-Sarawi, M.A., Landsberg, J., Durendez, R., Al-Marzouk, A. and Al-Zenki, S. (2002). Characterization of β-hemolytic group B *Streptococcus agalactiae* in cultured seabream, *Sparus auratus* and mullet, *Liza klunzingeri* in Kuwait. *Journal of Fish Diseases* 25: 505-513.

Evans, J.J., Pasnik, D.J., Klesius, P.H. and Shoemaker, C.A. (2006). Identification and epidemiology of *Streptococcus iniae* and *S. agalactiae* in tilapia, *Oreochromis* spp. *International Symposium on tilapia in Aquaculture 7.* Charles Town, WV, USA, American Tilapia Association. Pp. 25-42.

Holt, J.G., Krieg, N.R., Sheath, P.A., Staley, J.T. and William, S.T. (1994). *Bergey's Manual of Determination Bacteriology* (9th). Philadelphia: Williams & Wilkins.

Mian, G.F., Godoy, D.T., Lea, C.A.G., Yuhara, T.Y., Costa, G.M. and Figueiredo, H.C.P. (2009). Aspects of the natural history and virulence of *Streptococcus agalactiae* infection in Nile tilapia. *Veterinary Microbiology* 136: 180-183.

Najiah, M., Lee, S.W., Nadirah, M., Ruhil, H., Lee, K.L., Wendy, W., Amal, M.N.A., Basiriah, M.K. and Siti-Zahrah, A. (2009). Streptococcosis in red hybrid tilapia (*Oreochromis niloticus*) commercial farms in Malaysia. *Aquaculture Research* 40: 630-632.

Plumb, J.A., Schachte, J.H., Gaines, J.L., Peltier, W. and Carrol B. (1974). *Streptococcus* sp. from marine fishes along the Alabama and northwest Florida coast of the Gulf of Mexico. *Transactions of the American Fisheries Society* 103: 358-361.

Robinson, J.A. and Meyer, F.P. (1966). Streptococcal fish pathogen. *Journal of Bacteriology* 92: 512.

Shoemaker, C.A. and Klesius, P.H. (1997). Streptococcal disease problem and control: A review. Tilapia Aquaculture Ithaca, NY, USA. Northwest Regional Aquaculture Engineering Service. Pp. 671-680.

Shoemaker, C.A., Evans, J.J. and Klesius, P.H. (2000). Density and doses: factors affecting mortality of *Streptococcus iniae* infected tilapia (*Oreochromis niloticus*). *Aquaculture* 188: 229-235.

Skowron, R. (2010). The thermocline layer in the thermal water structure of selected Polish lakes. *Limnological Review* 7: 161-169.

Suanyuk, N., Kong, F., Ko, D., Gwendolyn, L., Gilbert, G.L. and Supamattaya, K. (2008). Occurrence of rare genotypes of *Streptococcus agalactiae* in cultured red tilapia *Oreochromis* sp. and Nile tilapia *O. niloticus* in Thailand - Relationship to human isolates? *Aquaculture* 284: 35-40.

Yanong, R.P.E. and Francis-Floyd, R. (2002). Streptococcal infections of fish. Report from University of Florida. Series from the Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Zulkafli, A.R. and Ahmad, A.O. (2000). *Fish Fauna in Kenyir Lake*. Percetakan Nasional Malaysia Berhad.

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## REFERENCES

Ali, A., Hassan, D., Saleha, A.A., Siti-Khairani, B. and Milud, A. (2010). *Streptococcus agalactiae* the etiological agent of mass mortality in farmed red tilapia (*Oreochromis* sp.) *Journal of Animal and Veterinary Advances* 9(20): 2640-2646.

Almazan, G. and Boyd, C.E. (1978). An evaluation of Secchi disk visibility for estimating plankton densities in fish ponds. *Hydrobiologia* 65: 601-608.

Amal, M.N.A., Zamri-Saad, M., Siti-Zahrah, A., Zulkafli, R., Misri, S., Nur-Nazifah, M. and Shahidan, H. (2010a). Prevalence of *Streptococcus agalactiae* in tilapia kept in different water bodies. *Online Journal of Veterinary Research* 14(2): 153-162.

Amal, M.N.A., Zamri-Saad M., Zulkafli A.R., Siti-Zahrah A., Misri S., Ramley B., Shahidan H. and Sabri M.Y. (2010b). Water thermocline confirms susceptibility of tilapia cultured in lakes to *Streptococcus agalactiae*. *Journal of Animal and Veterinary Advances* 9(22): 2811-2817.

Bromage, E.S. and Owens, L. (2002). Infection of barramundi *Lates calcarifer* with *Streptococcus iniae*: Effect of different routes of exposure. *Diseases of Aquatic Organisms* 52: 199-205.

Dodson, S.V., Maurer, J.J. and Shotts, E.B. (1999). Biochemical and molecular typing of *Streptococcus iniae* isolated from fish and human cases. *Journal of Fish Diseases* 22: 331-336.

Evans, J.J., Klesius, P.H., Gilbert, P.M., Shoemaker, C.A., Al-Sarawi, M.A., Landsberg J., Durendez, R., Al-Marzouk, A. and Al-Zenki, S. (2002). Characterization of β-hemolytic group B *Streptococcus agalactiae* in cultured seabream, *Sparus auratus* and mullet, *Liza klunzingeri*, in Kuwait. *Journal of Fish Diseases* 25: 505-513.

Firdaus-Nawi, M., Noraini, O., Sabri, M.Y., Siti-Zahrah, A., Zamri-Saad, M. and Latifah, H. (2011). The effects of oral vaccination of *Streptococcus agalactiae* on stimulating Gut-Associated Lymphoid Tissues (GALTs) in tilapia (*Oreochromis* sp.). *Pertanika Journal of Tropical Agricultural Science* 34(1): 137-143.

Garcia, J.C., Klesius, P.H., Evans, J.J. and Shoemaker, C.A. (2008). Non infectivity of cattle *Streptococcus agalactiae* in Nile tilapia (*Oreochromis niloticus*) and channel catfish (*Ictalurus punctatus*). *Aquaculture* 281: 151-154.

Gonzalez-Rey, C., Belin, A.-M., Jorbeck, H., Norman, M., Krovacek, K., Henriques, B., Kallenius, G. and Svenson, S.B. (2003). RAPD-PCR and PFGE as tools in the investigation of an outbreak of beta-haemolytic Streptococcus group A in a Swedish hospital. *Comparative Immunology, Microbiology and Infectious Diseases* 26: 25-35.

Holt, J.G., Krieg, N.R., Sheath, P.A., Staley, J.T. and William, S.T. (1994). Bergey's Manual of Determination Bacteriology. Philadelphia: Williams & Wilkins.

Kitao, T., Aoki, T. and Iwata, K. (1979). Epidemiological study on streptococcosis of cultured yellowtail (*Setiola quinqueradiafa*)-I. Distribution of *Streptococcus* sp. in seawater and muds round yellowtail farms. *Bulletin of the Japanese Society for the Science of Fish* 45(5): 67-572.

Malathum, K., Singh, K., Weinstock, G. and Murray, B. (1998). Repetitive sequence-based PCR versus pulsed-field gel electrophoresis for typing of *Enterococcus faecalis* at the subspecies level. *Journal of Clinical Microbiology* 36: 211-215.

Mian, G.F., Godoy, D.T., Leal, C.A.G., Yuhara, T.Y., Costa, G.M. and Figueiredo H.C.P. (2009). Aspects of the natural history and virulence of *Streptococcus agalactiae* infection in Nile tilapia. *Veterinary Microbiology* 136: 180-183.

Najiah, M., Lee, S.W., Nadirah, M., Ruhil, H., Lee, K.L., Wendy, W., Amal, M.N.A., Basiriah, M.K. and Siti-Zahrah, A. (2009). Streptococcosis in red hybrid tilapia (*Oreochromis niloticus*) commercial farms in Malaysia. *Aquaculture Research* 40: 630-632.

Nguyen, H.T. and Kanai, K. (1999). Selective agars for the isolation of *Streptococcus iniae* from Japanese flounder, *Paralichthys olivaceus*, and its cultural environment. *Journal of Applied Microbiology* 86: 769-776.

Nguyen, H.T., Kanai, K. and Yoshikoshi, K. (2002). Ecological investigation of *Streptococcus iniae* isolated in cultured Japanese flounder, *Paralicthys olivaceus* using selective isolation procedure. *Aquaculture* 205: 7-17.

Pereira, U.P., Mian, G.F., Oliveira, I.C.M., Benchetrit, L.C., Costa, G.M. and Figueiredo, H.C.P. (2010). Genotyping of *Streptococcus agalactiae* strains isolated from fish, human and cattle and their virulence potential in Nile tilapia. *Veterinary Microbiology* 140: 186-192.

Salvador, R., Muller, E.E., Freitas, J.C., Leonhadt, J.H., Giordano, L.G.P. and Dias, J.A. (2005). Isolation and characterization of *Streptococcus* spp. group B in Nile tilapia (*Oreochromis niloticus*) reared in hapas nets and earth nurseries in the northen region of Parana State, Brazil. *Ciencia Rural* 35: 1374-1378.

Sayed Zain, M.H., Shafie, M.B.S and Ramisah, M.S (2009) Genetic variability of *Artemisia capillaris* (Wormwood capillary) by random amplified polymorphic DNA (RAPD) in Terengganu State, Malaysia. *African Journal of Biotechnology* 8(9): 1810-1814.

Suanyuk, N., Kanghear, H., Khongpradit, R. and Supamattaya, K. (2005). *Streptococcus agalactiae* infection in tilapia (*Oreochromis niloticus*). *Songklanakarin Journal of Science and Technology* 27: 307-319.

Suanyuk, N., Kong, F., Ko, D., Gilbert, G.L. and Supamattaya, K. (2008) Occurrence of rare genotypes of *Streptococcus agalactiae* in cultured red tilapia, *Oreochromis* sp. and Nile tilapia, *O. niloticus* in Thailand-Relationship to human isolates?. *Aquaculture* 284: 35-40.

Wan Bayani, W.O., Shafie M.S. and Zaleha, K. (2009). Study on genetic variability of *Cassidula aurisfelis* (snail) by random amplified polymorphic DNAs. *African Journal of Biotechnology* 8(22): 6117-6119.

Zamri-Saad, M., Amal, M.N.A. and Siti-Zahrah, A. (2010). Pathological changes in red tilapias (*Oreochromis* spp.) naturally infected by *Streptococcus agalactiae*. *Journal of Comparative Pathology* 143: 227-229.

Zulkafli, A.R., Amal, M.N.A. and Misri, S. (2009). The effect of water quality and fish sizes on the susceptibility of red tilapia in floating net cages to *Streptococcus agalactiae* infection. *Proceeding of Asian Pacific Aquaculture Conference*, 4-6th November 2009, PWTC Kuala Lumpur. P. 78.

## REFERENCES


Al-Harbi, A.H. (1994). First isolation of *Streptococcus* sp. from hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) in Saudi Arabia. *Aquaculture* 128: 195-201.

Al-Harbi, A.H. and Uddin, M.N. (2004). Seasonal variation in the intestinal bacterial flora of hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) cultured in earthen ponds in Saudi Arabia. *Aquaculture* 229: 37-44.

Ali, A. (1987). Status of Seabass, *Lates calcarifer* culture in Malaysia. In: J.W. Copland and D.L. Grey (Editors), Management of Wild and Cultured Sea Bass/Barramundi. ACIAR Proceedings, No. 20, Australian Centre for International Agricultural Research, Canberra. Pp. 165-167.

Ali, A., Hassan, D., Saleha, A.A., Siti-Khairani, B. and Milud, A. (2010). *Streptococcus agalactiae* the etiological agent of mass mortality in farmed red tilapia (*Oreochromis* sp.). *Journal of Animal and Veterinary Advances* 9 20: 2640-2646.

Allen, D.A., Austin, B. and Colwell, R.R. (1983). Numerical taxonomy of bacterial isolates associated with a freshwater fishery. *Journal of General Microbiology* 129: 2043- 2062.

Al-Marzouk, A., Duremdez, R., Yuasa, K., Sameer, A.Z., Al-Gharabally, H. and Munday, B. (2005). Fish kill of mullet *Liza klunzingeri* in Kuwait Bay: The role of *Streptococcus agalactiae* and the influence of temperature. *In* P. Walker, R. Lester and M.G. Bondad-Reantaso (eds). Diseases in Asian Aquaculture V. Fish Health Section, Asian Fisheries Society, Manila. Pp. 143-153.

Amal, M.N.A. (2007). Isolation and identification of *Streptococcus* spp. isolated from red tilapia (*Oreochromis niloticus*), Undergraduate thesis, University Malaysia Terengganu.

Ang, K.J., Gopinath, N. and Chua, T.E. (1989). The status of introduced fish species in Malaysia, in S.S. de Silva (ed.) Exotic Aquatic Organism in Asia, Asian Fisheries Society Special Publication No. 3. Pp. 71-82.

Agnew, W. and Barnes, A.C. (2007). *Streptococcus iniae*: an aquatic pathogen of global veterinary significance and a challenging candidate for reliable vaccination. *Veterinary Microbiology* 122: 1-15.

Arulampalam, P., Yusoff, F.M., Shariff, M., Law, A.T. and Srinivasa Rao, P.R (1998). Water quality and bacterial populations in a tropical marine cage culture farm. *Aquaculture Research* 29: 617-624.

Balarin, J.D. and Hutton, J.P. (1979). *Tilapia: A Guide to their Biology and Culture in Africa*. University of Stirling, Stirling, United Kingdom.

Bhujel, R.C. (2000). A review of strategies for the management of Nile tilapia broodfish in seed production systems, especially in hapa-based systems. *Aquaculture* 181: 37-59.

Bowser, P.R., Wooster, G.A., Getchell, R.G. and Timmons, M.B. (1998). *Streptococcus iniae* infection of tilapia *Oreochromis niloticus* in a recirculation production facility. *Journal of World Aquaculture Society* 29: 335-339.

Boyd, E.C. and Tucker, C.S. (1998). *Pond Aquaculture Water Quality Management*. Kluwer Academic Publisher.

Bromage, E.S., Thomas, A. and Owens, L. (1999). *Streptococcus iniae*, a bacterial infection in barramundi *Lates calcarifer*. *Diseases of Aquatic Organisms* 36: 177-181.

Bromage, E.S. and Owens, L. (2002). Infection of barramundi *Lates calcarifer* with *Streptococcus iniae*: effects of different routes of exposure. *Diseases of Aquatic Organisms* 52: 199-205.

Bromage, S. and Owens, L. (2009). Environmental factors affecting the susceptibility of barramundi to *Streptococcus iniae*. *Aquaculture* 290: 224-228.

Bunch, E.C. and Bajerano, Y. (1997). The effect of environmental factors on the susceptibility of hybrid tilapia *Oreochromis niloticus* x *O. aureus* to streptococcosis. *Israeli Journal of Aquaculture* 49: 56-61.

Buck, J.D., Shepard, L.L., Bubucis, P.M., Spotte, S., Mcclave, K. and Cook, R.A. (1989). Microbiological characteristics of white whale (*Delphinapterus leucas*) from capture through extended captivity. *Canadian Journal of Fisheries and Aquatic Sciences* 46: 1914-1921.

Chang, P.H. and Plumb, J.A. (1996). Histopathology of experimental *Streptococcus sp.* infection in tilapia, *Oreochromis niloticus* (L.) and channel catfish, *Ictalurus punctatus* (Rafinesque). *Journal of Fish Diseases* 19: 235-241.

Chinabut, S. (2002). A case study of isopod infestation in tilapia cage culture in Thailand. In: Arthur, J.R., Phillips, M.J., Subasinghe, R.P., Reantaso, M.B. and MacRae, I.H. (Eds) *Primary Aquatic Animal Health Care in Rural, Small-scale, Aquaculture Development*. FAO Fisheries Technical Paper No. 406, FAO, Rome. Pp. 201-202.

Chun, S,-K. and Sohn, S.-G. (1985). Characteristics of *Flexibacter columnaris* isolated from tilapia (*Tilapia* sp.). *Bulletin of the Korean Fisheries Society* 18: 369-373.

Colorni, A., Diamant, A., Eldar, A., Kvitt, H. and Zlotkin, A. (2002). *Streptococcus iniae* infections in Red Sea cage cultured and wild fish. *Diseases of Aquatic Organisms* 49: 165-170.

Coward, K. and Bromage, N.R. (2000). Reproductive physiology of female tilapia broodstocks. *Reviews in Fish Biology and Fisheries* 10: 1-25.

de Ocampo, A.A. and Camberos, L.O. (1998). Histopatologica de la respuesta de la tilapia (*Oreochromis* sp.) a una infeccion mixta por myxosporidios. Estudio en un caso natural. *Veterinaria Mexico* 29: 213-216.

DOF (Department of Fisheries of Malaysia), (2010). Annual Fisheries Statistics 2010. Pp. 85.

Duremdez, R., Al-Marzouk, A., Qasem, J.A., Al-Harbi, A. and Gharabally, A. (2004). Isolation of *Streptococcus agalactiae* from cultured silver pomfret, *Pampus argenteus* (Euphrasen), in Kuwait. *Journal of Fish Diseases* 27: 307-310.

El Aamri, F., Padilla, D., Acosta, F., Caballero, M.J., Roo, J., Bravo, J., Vivas, J. and Real, F. (2010). First report of *Streptococcus iniae* in red porgy, *Pagrus pagrus (*L.). *Journal of Fish Diseases* 33: 901-905.

Eldar, A., Bejerano, Y., Livoff, A., Horovitcz, A. and Bercovier, H. (1995). Experimental streptococcal meningo-encephalitis in cultured fish. *Veterinary Microbiology* 43: 33-40.

Eldar, A. and Ghittino, C. (1999a). *Lactococcus garvieae* and *Streptococcus iniae* infections in rainbow trout *Oncorhynchus mykiss*: similar, but different diseases. *Diseases of Aquatic Organisms* 36: 227-231.

Eldar, A., Perl, S., Frelier, P.F. and Bercovier, H. (1999b). Red drum *Sciaenops ocellatus* mortalities associated with *Streptococcus iniae* infection. *Diseases of Aquatic Organisms* 36: 121-127.

El-Gohary, M.S.A. (2004). Effect of some organo-phosphorus compounds on some cultured fresh-water fish. PhD thesis, University of Alexandria, Alexandria. Egypt.

Elliott, J.A., Facklam, R.R. and Richter, C.B. (1990). Whole-cell protein patterns of nonhemolytic group B, type Ib, streptococci isolated from humans, mice, cattle, frogs and fish. *Journal of Clinical Microbiology* 28: 628-630.

El-Sayed, A.-F.M. (2006). *Tilapia Culture*. Oceanography Department, Faculty of Science, Alexandria University, Egypt. CABI Publishing.

El-Sharouny, H.M. and Badran, R.A.M. (1995). Experimental transmission and pathogenicity of some zoosporic fungi to tilapia fish. *Mycopathologia* 132: 95-103.

Evans, J.J., Klesius, P.H., Gilbert, P.M., Shoemaker, C.A., Al Sarawi, M.A., Landsberg, J., Duremdez, R., Al Marzouk, A. and Al Zenki, S. (2002). 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* 25: 505-513.

Evans, J.J., Pasnik D.J., Klesius, P.H. and Al-Ablani, S. (2006a). First report of *Streptococcus agalactiae* and *Lactococcus garvieae* from a wild bottlenose dolphin (*Tursiops truncates*). *Journal of Wildlife Diseases* 42(3): 561-569.

Evans, J.J., Pasnik, D.J., Klesius, P.H. and Shoemaker, C.A. (2006b). Identification and epidemiology of *Streptococcus iniae* and *S. agalactiae* in tilapia, *Oreochromis* spp. *International Symposium on tilapia in Aquaculture 7.* Charles Town, WV, USA, American Tilapia Association. Pp. 25-42.

Everitt, S. and Leung, C.-F.A. (1999). Fish disease in Hong Kong. Paper presented at the Fourth Symposium on Diseases in Asian Aquaculture: Aquatic Animal Health for Sustainability, 22-26 November, Cebu City, Philippines.

FAO (Food and Agricultural Organization of the United Nation), (2004). Fishstat Plus. FAO, Rome.

FAO (Food and Agricultural Organization of the United Nation), (2009). FAO yearbook. Fisheries and aquaculture statistic. FAO, Rome.

FAO (Food and Agricultural Organization of the United Nation), (2010). The State of World Fisheries and Aquaculture. FAO, Rome.

Filho, C.I., Muller, E.E., Pretto-Giordano, L.G. and Bracarense, A.P.F.R.L. (2009). Histological findings of experimental *Streptococcus agalactiae* infection in Nile tilapia (*Oreochromis niloticus*). *Brazilian Journal of Veterinary Pathology* 2(1): 12-15.

Fryer, G. and Iles, T.D. (1972). *The Cichlid Fishes of the Great Lakes of Africa: Their Biology and Evolution*. T.F.H. Publishing, Neptune City, New Jersey.

Garcia, P.G.L, Muller, E.E., Klesius, P.H. and da Silva, V.G. (2010a). Efficacy of an experimentally inactivated *Streptococcus agalactiae* vaccine in Nile tilapia (*Oreochromis niloticus*) reared in Brazil. *Aquaculture Research* 41: 1539-1544.

Garcia, P.G.L., Müller, E.E., de Freitas, J.C. and da Silva, V.G. (2010b). Evaluation on the pathogenesis of *Streptococcus agalactiae* in Nile tilapia (*Oreochromis niloticus*). *Brazilian Achieves of Biology and Technology* 53(1): 87-92.

Gilmour, A., McCallum, M.F. and Allan, M.C. (1976). A study of the bacterial types occurring on the skin and in the intestine of farmed plaice (*Pleuronectes platessa* L.). *Aquaculture* 7: 161-172.

Gopinath, N. and Chin, C. W. (1998). Marine Finfish Culture. In N. Gopinath and T. Singh (Editors). Aquaculture Practices in Malaysia. Malaysian Fisheries Society Occasional Publication No.9. UPM, Serdang, Selangor. Pp. 81-86.

Guillen, I., Berlanga, J., Valenzuela, C.M. Morales, A., Toledo, J., Estrada, M.P., Puentes, P., Hayes, O. and de la Fuente, J. (1999). Safety evaluation of transgenic tilapia with accelerated growth. *Marine Biotechnology* 1: 2-14.

Hendrick, R.P., Fryer, J.L., Chen, S.N. and Kou, G.H. (1983). Characteristics of four birnaviruses isolated from fish in Taiwan. *Fish Pathology* 18: 91-97.

Henton, M.M., Zapke, O. and Basson, P.A. (1999). *Streptococcus phocae* infections associated with starvation in Cape fur seals. *Journal of the South African Veterinary Medical Association* 70: 98-99.

Hoshina, T., Sano, T. and Marimoto, Y. (1958). A Streptococcus pathogenic to fish, *Journal of the Tokyo University for Fisheries* 44: 57-58.

Huang, S.-L., Liao, I.-C. and Chen, S.-N. (2000). Induction of apoptosis in tilapia, *Oreochromis aureus* Steindachner, and in TO-2 cells by *Staphylococcus epidermidis*. *Journal of Fish Diseases* 23: 363-368.

Hussin, M.A., Nik Daud, N.S. and Nik Razali, N.L. (1996). Natural spawning and larval rearing of tiger grouper, *Epinephalus fuscoguttatus* (Forskal). A preliminary result. Paper presented at the 5th Fisheries Research Institute Conference. 8-10 July 1997. Fisheries Research Institute, Penang, Malaysia.

Kim, J.H., Gomez, D.K., Choresca, C.H. and Park, S.C. (2007). Detection of major bacterial and viral pathogens in trash fish used to feed cultured flounder in Korea. *Aquaculture* 272: 105-110.

Klesius, P.H., Shoemaker C.A. and Evans J.J. (2000). Efficacy of single and combined *Streptococcus iniae* isolate vaccine administered by intraperitoneal and intramuscular routes in tilapia (*Oreochromis niloticus*). *Aquaculture* 188: 237-246.

Klesius, P.H., Shoemaker, C.A. and Evans, J.J. (2008). Streptococcus: A Worldwide Fish Health Problem. *8th International Symposium on Tilapia in Aquaculture*, Cairo, Egypt. Pp. 83-107.

Kuperman, B.I., Matey, V.E. and Barlow, S.B. (2002). Flagellate *Crytobia branchialis* (Bodonida: Kinetoplastida), ectoparasite of tilapia from the Salton Sea. *Hydrobiologia* 473: 93-102.

Lammer, C.H., Abdulmawjood, A. and Weiss, R. (1998). Properties of serological group B streptococci of dogs, cats and monkey origin. *Journal of Clinical Microbiology* 45: 561-566.

Lancefield, R.C. (1933). A serological differentiation of human and other groups of hemolytic streptococci. *Journal of Experimental Medicine* 59: 571-591.

Lautenslager, G.T. (1986). Cichlid infectious agent virus: a characterization of a new rhabdovirus of cichlid fish. PhD thesis, Lehigh University, Bethlehem, Pennsylvania.

Lightner, D., Redman, R., Mohney, L., Dickenson, G. and Fitzsimmons, K. (1988). Major diseases encountered in controlled environment culture of tilapia in fresh- and brackish water over a three year period in Arizona. In: Pullin, R.S.V., Bhukaswan, T., Tonguthai, K. and Maclean, J.L. (Eds) *Proceedings of the Second International Symposium on Tilapia in Aquaculture*. ICLARM Conference Proceeding No. 15, Department of Fisheries, Bangkok, Thailand, and ICLARM, Manila, Philippines. Pp. 111-116.

Lim, C. and Klesius, P.H. (2001). Influence of dietary levels of folic acid on growth response and resistance of Nile tilapia *Oreochromis niloticus* to *Streptococcus iniae*. In: *Sixth Asian Fisheries Forum. Book of Abstracts*. Asian Fisheries Society, Quezon City, Philippines. Pp. 150.

Little, D.C., Macintosh, D.J. and Edward, P. (1993). Improving spawning synchrony in the Nile tilapia, *Oreochromis niloticus* (L*). Aquaculture and Fisheries Management* 24: 399-405.

Manna, G.K. and Sadhukhan, A. (1991). Genotoxic potential of the spores of three species of fungi experimented on cichlid fish, *Oreochromis mossambicus*. *Kromosomo* 2: 2129-2134.

Mian, G.F., Godoy, D.T., Leal, C.A.G., Yuhara, T.Y., Costa, G.M. and Figueiredo H.C.P. (2009). Aspects of the natural history and virulence of *Streptococcus agalactiae* infection in Nile tilapia. *Veterinary Microbiology* 136: 180-183.

Miyashita, T. (1984). *Pseudomonas fluorescens* and *Edwardsiella tarda* isolated from diseased tilapia. *Fish Pathology* 19: 45-50.

Najiah, M., Lee, S.W., Nadirah, M., Ruhil, H., Lee, K.L., Wendy, W., Amal, M.N.A., Basiriah, M.K. and Siti-Zahrah, A. (2009). Streptococcosis in red hybrid tilapia (*Oreochromis niloticus*) commercial farms in Malaysia. *Aquaculture Research* 40: 630-632.

Nguenga, D. (1998). A note on infestation of *Oreochromis niloticus* with *Trichodina* sp. and *Dactylogyrus* sp. In: Pullin, R.S.V., Bhukaswan, T., Tonguthai, K. and Maclean, J.L. (Eds) *Proceedings of the Second International Symposium on Tilapia in Aquaculture*. ICLARM Proceedings No. 15, Department of Fisheries, Bangkok, Thailand, and ICLARM, Manila, Philippines. Pp. 117-119.

Nguyen, H.T., Kanai, K. and Yoshikoshi, K. (2002). Ecological investigation of *Streptococcus iniae* isolated in cultured Japanese flounder, *Paralicthys olivaceus* using selective isolation procedure. *Aquaculture* 205: 7-17.

Nur-Nazifah, M., Sabri, M.Y., Amal, M.N.A., Latifah, H. and Siti-Zahrah, A. (2009). Isolation and identification of *Streptococcus agalactiae* from *Oreochromis* spp. at Broga, Semenyih, Malaysia. *Proceeding of the 21st Malaysian Veterinary Annual Conference*, 8th-9th August 2009, Port Dickson, Malaysia. Pp. 360-362.

Okaeme, A.N., Obiekezie, A., Okojie, P.U. and Agbontale, J.J. (1989). Histopathology of normal and infected organs of tilapia by *Myxobolus ovariae*. In: *Annual Report,* Appendix E. East African Freshwater Fish Research Organization, Nairobi, Kenya. Pp. 50-58.

Ortega, C., Muzquiz, J.L., Fernandez, A., Ruiz, I., De Blas, I., Simon, M.C. and Alonso, J.L. (1996). Water quality parameters associated with *Aeromonas* spp.-affected hatcheries. *Veterinary Research* 27: 553-560.

Paperna, I. (1974). Lymphocystis in fish from East African lakes. *Journal of Wildlife Diseases* 9: 331-335.

Paperna, I. and Smirnova, M. (1997). *Branchiomyces*-like infection in a cultured tilapia (*Oreochromis* hybrid, Cichlidae). *Diseases of Aquatic Organisms* 31: 233-238.

Pillay, T.V.R. (1990). *Aquaculture Principles and Practices*. Fishing News Books, Blackwell Science, Oxford, United Kingdom.

Plumb, J.A., Schachte, J.H., Gaines, J.L., Peltier, W. and Carrol, B. (1974). *Streptococcus* sp. from marine fishes along the Alabama and northwest Florida coast of the Gulf of Mexico. *Transactions of the American Fisheries Society* 103: 358-361.

Popma, T. and Masser, M. (1999). *Tilapia Life Story and Biology*. Southern Regional Aquaculture Center Publication No. 283.

Roberts, R.J. and Sommerville, C. (1982) Diseases of tilapia. In: Pullin, R.S.V. and Lowe-McConnell, R.H. (eds) The Biology and Culture of Tilapias. ICLARM Conference Proceedings No. 7, ICLARM, Manila, Philippines. Pp. 247-264.

Robinson, J.A. and Meyer, F.P. (1966). Streptococcal fish pathogen. *Journal of Bacteriology* 92: 512.

Saeed, M.O. (1993). Efforts to control outbreaks of diseases among cultured fish in Kuwait. In: *Proceedings of the First International Aquaculture Symposium: Technologies and Investment Opportunities*. Ministry of Agriculture and Waters, Riyadh, Saudi Arabia. Pp. 400-409.

Saeed, M.O. and Al-Thobaiti, S.A. (1997). Gas bubble disease in farmed fish in Saudi Arabia. *Veterinary Record* 140: 682-684.

Salvador, R., Muller, E.E., Freitas, J.C., Leonhadt, J.H., Giordano, L.G.P. and Dias, J.A. (2005). Isolation and characterization of *Streptococcus* spp. group B in Nile tilapia (*Oreochromis niloticus*) reared in hapas nets and earth nurseries in the northen region of Parana State, Brazil. *Ciencia Rural* 35: 1374-1378.

Shoemaker, C.A. and Klesius, P.H. (1997). Streptococcal disease problem and control: A Review. *Tilapia Aquaculture Ithaca*, NY, USA. Northwest Regional Aquaculture Engineering Service. Pp. 671-680.

Shoemaker, C.A., Evans, J.J. and Klesius, P.H. (2000). Density and doses: factors affecting mortality of *Streptococcus iniae* infected tilapia (*Oreochromis niloticus*). *Aquaculture* 188: 229-235.

Shoemaker, C.A., Klesius, P.H. and Evans, J.J. (2001). Prevalence of *Streptococcus iniae* in tilapia, hybrid striped bass, and channel catfish on commercial fish farms in the United States. *American Journal of Veterinary Research* 62(2): 174-177.

Siti-Zahrah, A., Misri, S., Padilah, B., Zulkafli, R., Kua, B.C., Azila, A. and Rimatulhana, R. (2004). Pre-disposing factors associated with outbreak of Streptococcal infection in floating cage-cultured red tilapia in reservoirs. *Abstract of the 7th Asian Fisheries Forum 04, The Triennial Meeting of The Asian Fisheries Society,* 30th Nov-4th Dec 2004, Penang, Malaysia. Pp. 129.

Siti-Zahrah, A., Padilah, B., Azila, A., Rimatulhana, R. and Shahidan, H. (2005). Multiple streptococcal species infection in cage-cultured red tilapia, but showing similar clinical sign. *Proceedings of the Sixth Symposium on Disease in Asian Aquaculture* (Eds. by Bondad-Reantaso, M.G., Mohan, C.V., Crumlish. M. and Subasinghe, R.P.) Colombo, Sri Lanka. Pp. 332-339.

Siti-Zahrah, A., Zamri-Saad, M., Zulkafli, A.R., Sabri, M,Y., Amal, M.N.A., Misri, S., Norazlan, G.W. and Nur-Nazifah, M. (2009). Prevalence and significant of Streptococcosis in cage cultured red tilapia of West Malaysia. *Proceeding of Asian Pacific Aquaculture Conference,* 4-6th November of 2009, PWTC Kuala Lumpur, Malaysia. Pp. 49.

Skowron, R. (2010). The thermocline layer in the thermal water structure of selected Polish lakes. *Limnological Review* 7: 161-169.

Soliman, A.K. and Wilson, R.P. (1992). Water-soluble vitamin requirements of tilapia. 1. Pantothenic acid requirement of blue tilapia, *Oreochromis aureus*. *Aquaculture* 104: 121-126.

Suanyuk, N., Kanghear, H., Khongpradit, R. and Supamattaya, K. (2005). *Streptococcus agalactiae* infection in tilapia (*Oreochromis niloticus*). *Songklanakarin Journal of Science and Technology* 27 (1): 307-319.

Suanyuk, N., Kong, F., Ko, D., Gilbert, G.L. and Supamattaya, K. (2008). Occurrence of rare genotypes of *Streptococcus agalactiae* in cultured red tilapia *Oreochromis* sp. and Nile tilapia *O. niloticus* in Thailand-Relationship to human isolates?. *Aquacultur*e 284: 35-40.

Swenshon, M., Lammer, C. and Siebert, U. (1998). Identification and molecular characterization of beta-hemolytic streptococci isolated from harbour porpoises (*Phocoena phocoena*) of the North and Baltic Seas. *Journal of Clinical Microbiology* 36: 1902-1906.

Thalathiah, S., and Hamilah, H. (1986). Induced spawning of *Pangasius sutchi* (Fowler) using an analog of luteinizing-releasing harmone and homoplastic pituitary extract. In: Maclean, J.L., L.B. Dizon, L.V. Hosillos (Eds.), The First Asian fisheries Forum, Manila, Philippines, 26-31 May, 1986. Asian Fisheries Society. Pp. 687-688.

Thalathiah, S. (1986). Induced spawning of *Clarias macrocephalus* (Gunther). In: Maclean, J.L., L.B. Dizon, L.V. Hosillos (Eds.), The First Asian Fisheries Forum, Manila, Philippines, 26-31 May, 1986. Asian Fisheries Society. Pp. 683-688.

Thalathiah, S. (1988). First successful attempt to induce breed, baung river catfish, *Mystus nemurus* (C&V) at Batu Berendam. In: Proceedings of the 11th Annual Conference, Malaysian Society of Animal Production. Pp. 53-55.

Thalathiah, S., and Ibrahim, T. (1992). Seed production of *Clarias gariepinus* (Burchell). In: Ho. Y.W. et at., (Eds.). Proceedings of the National Intensification of Research in Priority Areas Seminar, Kuala Lumpur, 6-11 January, 1992, Volume II. Pp. 239-240.

Trewaves, E. (1982). Tilapias: Taxonomy and speciation. In. Pullin, R.V.S. and Lowe-McConnell, R.D. (Eds) *The Biology and Culture of Tilapias*. ICLRAM Conference Proceedings No. 7, ICLRAM, Manila, Philippines. Pp. 3-13.

Trust, T.J. and Sparrow, R.A.H. (1974). The bacterial flora in the alimentary tract of freshwater salmonid fishes. *Canadian Journal of Microbiology* 20: 1219-1228.

Turner, G.F. and Robinson, R.L. (2000). Reproductive biology, mating sytems and parental care. In: Beverigde, M.C.M. and McAndrew, B.J. (Eds) *Tilapias: Biology and Exploitation*. Kluwer Academic Publisher, Dordrecht/Boston/London. Pp. 33-58.

Wang, Z-G. and Xu, B-H. (1985). Studies on the pathogenic bacteria of the 'rotten-skin' diseases of the Nile tilapia (*Tilapia nilotica*). *Journal of Fisheries of China* 9: 217-221.

Wibawan, I.W.T., Lautrou, Y. and Lammler, C. (1991). Antibiotic resistance patterns and pigment production of streptococci of serological group B isolated from bovines and humans. *Zentralbl Veterinarmed B*. 38: 731-736.

Wibawan, I.W.T., Lammer, C.H. and Smola, J. (1993). Properties and type antigen patterns of group B Streptococcus isolates from dog and nutrias. *Journal of Clinical Microbiology* 31: 762-764.

Wolf, J.C. and Smith, S.A. (1999). Comparative severity of experimentally induced mycobacteriosis in striped seabass *Morone saxatilis* and hybrid tilapia *Oreochromis* spp. *Diseases of Aquatic Organisms* 38: 191-200.

Yanong, R.P.E. and Francis-Floyd, R. (2002). Streptococcal infections of fish. Report from University of Florida. Series from the Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Yildirim, A,. Lammer, O. and Weiss, R. (2002). Identification and characterization of *Streptococcus difficile* is a nonhemolytic group B, type Ib *Streptococcus. International Journal of Systemic Bacteriology* 47: 81-85.

Zlotkin, A., Hershko, H. and Eldar, A. (1998). Possible transmission of *Streptococcus iniae* from wild to cultured marine fish. *Applied and Environmental Microbiology* 64: 4065-4067.

Zulkafli, A.R., Amal, M.N.A. and Misri, S. (2009). The effect of water quality and fish sizes on the susceptibility of red tilapia in floating net cages to *Streptococcus agalactiae* infection. *Proceeding of Asian Pacific Aquaculture Conference,* 4-6th November of 2009, PWTC Kuala Lumpur. Pp. 78.